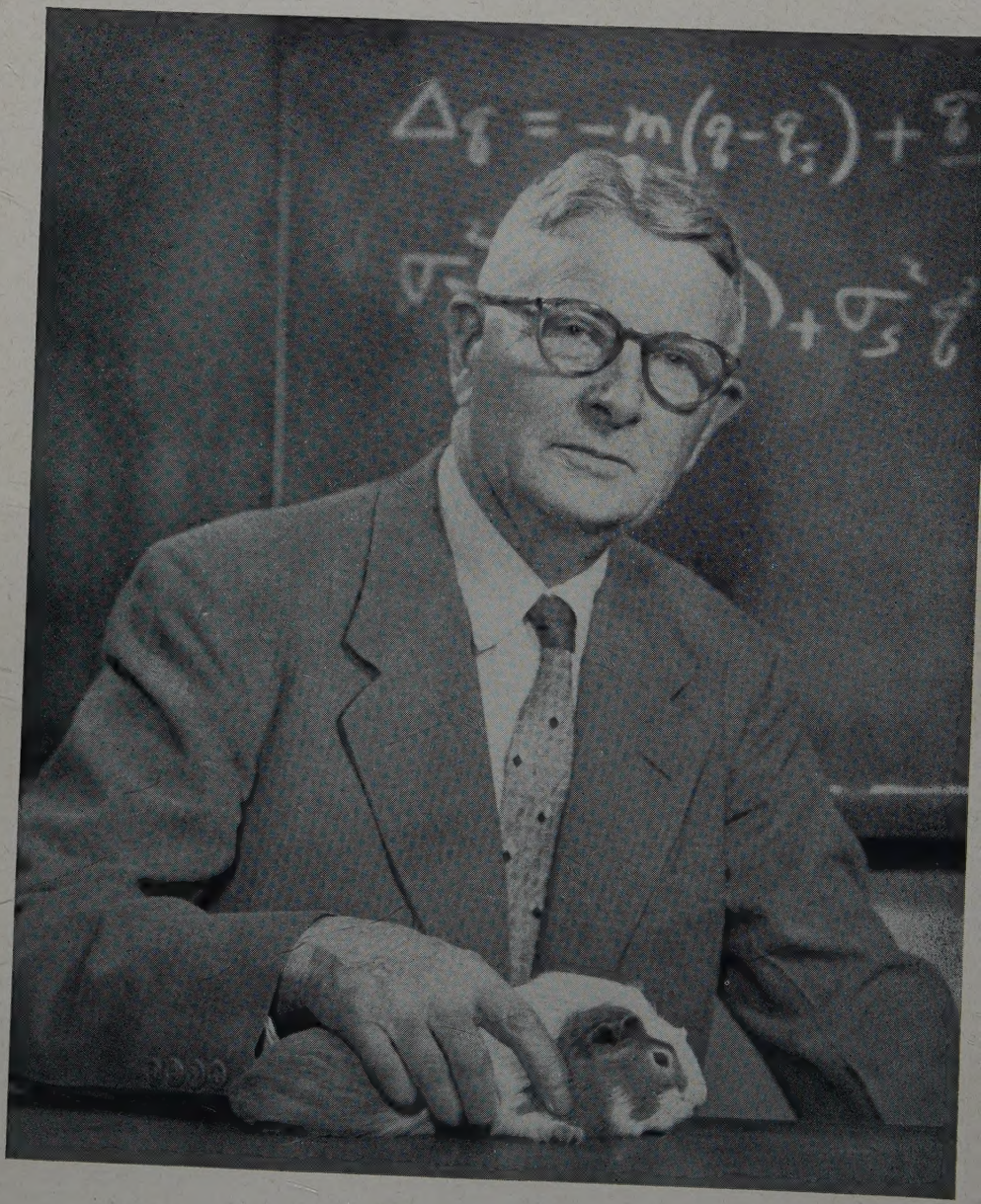
8/2017
STRAND PRICE
$2.00

PROCEEDINGS OF THE
X INTERNATIONAL CONGRESS OF GENETICS



Sewall Wright, President

Proceedings of the

X INTERNATIONAL CONGRESS OF GENETICS

Comptes rendus du

Xe CONGRÈS INTERNATIONAL DE GÉNÉTIQUE

August 20–27, 1958

McGILL UNIVERSITY, MONTREAL

President

SEWALL WRIGHT

Vice-Presidents

C. BARIGOZZI
A. MÜNTZING
C. PAVAN
L. S. PENROSE
Y. SINOTO
A. STURTEVANT
W. P. THOMPSON

Volume I: Papers, Lectures, Minutes, and Reports

University of Toronto Press, Toronto, Ontario, Canada

1959

Copyright, Canada, 1959
University of Toronto Press

Printed in Canada

Vice-Presidents

Professor C. Barigozzi

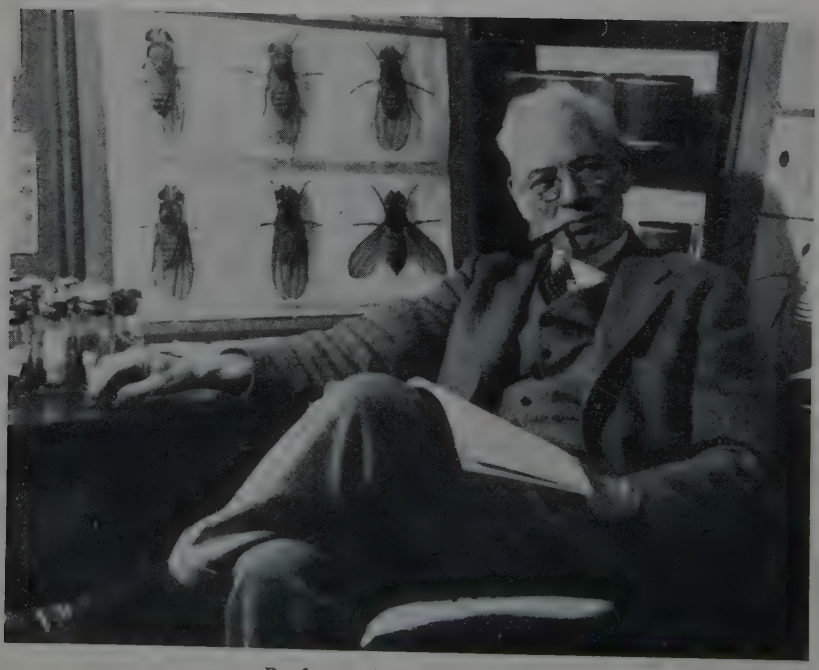
Professor A. Müntzing

Professor C. Pavan

Professor L. S. Penrose

Professor Y. Sinotô

Professor W. P. Thompson



Professor A. H. Sturtevant

CONGRESS COMMITTEES AND SPONSORS
COMITÉS ET PATRONS DU CONGRÈS

The Honorary Committee—Le Comité Honoraire

THE RIGHT HONOURABLE JOHN GEORGE DIEFENBAKER, P.C., Q.C., M.A., LL.B., LL.D., D.C.L., Prime Minister of Canada

THE HONOURABLE J. WALDO MONTEITH, P.C., F.C.A., Minister of National Health and Welfare, Ottawa

LT.-COL. THE HONOURABLE DOUGLAS S. HARKNESS, P.C., Minister of Agriculture, Ottawa

J. G. TAGGART, C.B.E., LL.D., Deputy Minister of Agriculture, Ottawa

THE HONOURABLE MAURICE L. DUPLESSIS, Premier, Province of Quebec

THE HONOURABLE LAURENT BARRÉ, Minister of Agriculture, Province of Quebec

HIS WORSHIP THE HONOURABLE SARTO FOURNIER, Mayor of the City of Montreal

F. CYRIL JAMES, Ph.D., D.Sc., D.de l'U., D.C.L., LL.D., F.R.S.C., Principal and Vice-Chancellor, McGill University

MONSEIGNEUR IRENÉE LUSSIER, P.D., L.Th., L.Ph., D.C.L., Rector of the University of Montreal

ANDREW STEWART, LL.D., D.Econ., President, University of Alberta; President, National Council of Canadian Universities

E. W. R. STEACIE, O.B.E., Ph.D., D.Sc., LL.D., D.de l'U., F.R.S.C., F.R.S., President, National Research Council

H. G. DION, Ph.D., Vice-Principal, Macdonald College

C. H. GOULDEN, Ph.D., LL.D., F.R.S.C., Director, Experimental Farms Service, Canada Department of Agriculture

K. W. NEATBY, Ph.D., LL.D., Director, Science Service, Canada Department of Agriculture

Sponsor—Patron

Genetics Society of America

Co-sponsors—Co-patrons

Genetics Society of Canada

Agricultural Institute of Canada
American Cancer Society
American Eugenics Society
American Genetic Association
American Society of Agronomy
American Society for Animal Production
American Society for Horticultural Science
American Society of Human Genetics
American Society of Naturalists
Society for the Study of Evolution

The Permanent International Committee for Genetics Congresses
Le Comité International Permanent des Congrès de Génétique

(The Genetics Section of the International Union of Biological Sciences)
(La Section Génétique de l'Union Internationale des Sciences Biologiques)

C. Barigozzi (Italy—*President*), I. M. Lerner (U.S.A. and Canada—*Secretary*), F. G. Brieger (Latin America), B. Ephrussi (France), Naomi Feinbrun-Dothan (Near East), E. Hadorn (Switzerland and Austria), S. C. Harland (Great Britain and Commonwealth), N. Parthasarathy (Southern Asia), Y. Sinotô (Japan), M. J. Sirks (Belgium and Netherlands), H. Stubbe (Germany), A. Tavčar (Southeastern Europe), M. Westergaard (Scandinavian Countries), A. de Zulueta (Spain and Portugal)

The General Organizing Committee—Le Comité Général d'Organisation

J. W. Boyes (*Chairman and General Secretary*), E. W. Caspari, M. Demerec, P. C. Mangelsdorf, H. B. Newcombe (*ex-officio for the Genetics Society of America*), C. P. Oliver (*ex-officio for the Genetics Society of America*), R. F. Peterson (*representing the Genetics Society of Canada*), F. J. Ryan, H. H. Smith, S. G. Smith, Norma F. Walker

The Exhibits Committee—Le Comité de l'Exposition

H. H. Smith (*Chairman*), N. Bayley, J. W. Boyes, J. L. Brewbaker, W. E. Heston, A. W. S. Hunter, G. I. Paul, A. M. Srb, W. Szybalski

The Finance Committees—Le Comité des Finances

Canadian Committee: S. G. Smith (*Chairman*), R. F. Peterson (*Vice-Chairman*), J. R. Beaudry, J. W. Boyes, R. Prunsch, J. Rousseau; *Corresponding members:* T. J. Arnason, C. J. Bishop, V. C. Brink, L. Chouinard, J. P. W. Gilman, C. G. Hickman, L. P. V. Johnson; *Montreal committee:* R. Prunsch (*Chairman*), J. R. Beaudry, E. R. Boothroyd, P. Couillard, R. P. Poirier

United States Committee: F. J. Ryan (*Chairman*), J. A. Moore (*Vice-Chairman*), W. D. McElroy, C. Pittendrigh, A. M. Srb, B. Wallace; *Corresponding members:* O. J. Eigsti, E. Gardner, E. L. Green, A. Hecht, I. Herskowitz, N. Horowitz, J. Lederberg, R. P. Levine, R. C. Lewontin, J. V. Neel, J. R. Preer, D. Schwartz, E. R. Sears, F. E. Stephens, C. Swanson, E. P. Volpe, R. P. Wagner

The Ladies Committee—Le Comité des Dames

Mrs. J. W. Boyes (*Chairman*), Mme. M. Cailloux, Mme. P. Dansereau, Mme. J. M. Demers, Mrs. F. C. Fraser, Mme. R. Gauthier, Mrs. W. F. Grant, Mrs. G. A. Grimson, Mrs. C. P. Leblond, Mrs. R. B. Malmo, Mrs. K. I. Melville, Dr. K. Metrakos, Mrs. J. R. Miller, Mrs. F. Morrison, Mrs. H. R. Murray, Mrs. H. S. Mitchell, Mrs. D. L. Peets, Mrs. H. R. Scott, Mrs. K. D. Sharma, Mrs. V. Sinclair, Mrs. H. Steppler, Mrs. E. C. Webster, Mrs. C. A. Winkler

The Local Committee—Le Comité Local

J. W. Boyes (*Chairman*), F. C. Fraser (*Co-chairman*), J. R. Beaudry, A. Blain, E. R. Boothroyd, Mrs. J. W. Boyes, R. I. Brawn, P. Dansereau, W. F. Grant, J. D. Metrakos, J. R Miller, G. I. Paul, Muriel V. Roscoe, H. Steppler

The Programme Committee—Le Comité du Programme

E. W. Caspari (*Chairman*), J. W. Boyes (*ex-officio*), L. Butler, M. Demerec, N. H. Giles, H. B. Glass, F. B. Hutt, D. F. Jones, J. D. Metrakos, W. P. Spencer, H. T. Stinson, B. Wallace; *Corresponding members:* T. J. Arnason, C. J. Bishop, R. A. Brink, V. C. Brink, J. Crow, T. Dobzhansky, L. C. Dunn, J. W. Gowen, A. Hollaender, F. H. Hull, E. B. Lewis, S. E. Luria, C. P. Oliver, H. Roman, G. L. Stebbins, C. Stern, C. Stormont, J. Unrau

The Publications Committee—Le Comité des Publications

Norma F. Walker (*Chairman*), J. R. Beaudry, L. Butler, G. M. Clark, Margaret E. Hatton, D. B. W. Reid, K. H. Rothfels, L. Tallan, Irene Uchida

The Travel Assistance Committee—Le Comité d'Assistance au Voyage

P. C. Mangelsdorf (*Chairman*), Harriet B. Creighton, A. W. S. Hunter, E. Mayr, R. P. Levine, F. J. Ryan, A. H. Sparrow

The Secretaries and Assistants—Les Secrétaires et Assistants

J. W. Boyes (*General Secretary*), Joan M. Robertson (*Executive Secretary*), May Macmillan, Florence Akst, Harriet Nussbaum, Mildred Meek, Merrille Feiner, Anne Tector, Joanne Ashworth, Evelyn Roy

CONTENTS

Records of the Inaugural Session 3
Records of Special Convocations during the Congress 10
Minutes of the Business Meeting and Concluding Ceremony 19
Open Letter to the Permanent International Committee for Genetics Congresses from the Soviet Delegation 28
The Story of the X International Congress of Genetics. J. W. BOYES 30

THE STRUCTURE OF GENETIC MATERIAL

Genetic Structure of the *Salmonella* Chromosome. M. DEMEREC 55
The Organization and Duplication of Genetic Material. J. HERBERT TAYLOR 63

ADVANCES IN HUMAN GENETICS

Genetic Aspects of Schizophrenic Psychoses. JAN A. BÖÖK 81
Etudes sur l'étiologie de la gémellité chez l'homme. MAURICE LAMY ET JEAN FRÉZAL 89
Some Recent Developments in Human Biochemical Genetics. H. HARRIS 100
Aspects of the Genetic Control of the Structure of the Hemoglobin Molecule. JAMES V. NEEL 108
Some Further Observations on Associations between Blood Groups and Disease. J. A. FRASER ROBERTS 120

CYTOGENETICS AND PLANT BREEDING

Cytogenetics and Wheat-breeding. JOHN UNRAU 129
Fertility and Morphological Variation in the Substitution and Restoration Backcrosses of the Hybrids, *Triticum vulgare* × *Aegilops caudata*. H. KIHARA 142
Basic Research in Plant and Animal Improvement. DONALD F. JONES 172
Genetics in Silviculture. C. SYRACH LARSEN 177

GENETICS IN ANIMAL BREEDING

Problems of Comparative Genetics in Mammals. H. NACHTSHEIM 187
Laboratory Breeding Experiments and Animal Improvement. ALAN ROBERTSON 199
On the Applications of Blood Groups in Animal Breeding. CLYDE STORMONT 206
Evidence from Twins on Variation in Growth and Production of Cattle. H. P. DONALD 225
The Genetics of Milk Yield. IVAR JOHANSSON 236

Mutation and Mutagenesis

Advances and Problems of Research in Mutations in the Applied Field. H. STUBBE 247
Mutations at Specific Loci in *Neurospora*. NORMAN H. GILES 261
Post-Irradiation Metabolism and the Timing of Ultraviolet-induced Mutations in Bacteria. EVELYN M. WITKIN 280
Metabolism and the Stability of Chromosomes. C. P. SWANSON, T. MERZ, AND N. COHN 300
The Mutation Theory Re-examined. H. J. MULLER 306

Physiological Genetics

Morphological and Physiological Aspects of Chromosomal Activities. C. PAVAN 321
Contribution to the Physiological and Biochemical Genetics of Pteridines and Pigments in Insects. ERNST HADORN 337
The Role of Chromosomal Loci in Enzyme Formation. J. R. S. FINCHAM 355
Immunogenetics. RAY D. OWEN 364
Cytoplasmic Inheritance and the Segregation of Plasmagenes. P. MICHAELIS 375

Darwin Centennial Symposium: Genetics in Evolution

Some Data on the Genetics Structure of Human Populations. L. L. CAVALLI-SFORZA 389
The Role of Heterozygosity in *Drosophila* Populations. BRUCE WALLACE 408
The Mesophragmatica Group as an Example of the Speciation Phenomena in *Drosophila*. DANKO BRNCIC 420
Gene Systems Regulating Characters of Ecological Races and Subspecies. JENS CLAUSEN 434
The Cytogenetic Basis of Speciation in Coleoptera. STANLEY G. SMITH 444

Special Lectures

A New Category of Chromosomes. ARNE MÜNTZING 453
Genetics and the Destiny of Man. THEODOSIUS DOBZHANSKY 468
Genetics, the Gene, and the Hierarchy of Biological Sciences. SEWALL WRIGHT 475

Panel Discussions

Panel on the Teaching of Genetics. Reported by BENTLEY GLASS, Moderator 493
A Discussion on *Drosophila* Terminology. Reported by K. BREHME-WARREN AND C. P. OLIVER 497

Members of the Congress 499

RECORDS OF THE INAUGURAL SESSION

THE INAUGURAL SESSION was held on August 20, 1958, in the open at the Percival Molson Memorial Stadium of McGill University. It commenced at 9:20 A.M. and closed at 11:00 A.M. Seated on the platform were Professor Sewall Wright, President of the Congress; the Honourable Henri Courtemanche, Secretary of State, Government of Canada; the Honourable Paul Dozois, Minister of Municipal Affairs, Government of the Province of Quebec; Mr. C. Hugh Hanson, representing His Worship Mayor Sarto Fournier of the City of Montreal; the following Vice-Presidents of the Congress: Professor C. Pavan, Professor A. Müntzing, Professor L. S. Penrose, Professor Y. Sinotô, Professor A. H. Sturtevant, Professor W. P. Thompson; Professor C. Barigozzi, President of the Permanent International Committee for Genetics Congresses and a Vice-President of the Congress; Professor C. P. Oliver, President of the Genetics Society of America; Dr. C. J. Bishop, President of the Genetics Society of Canada; and Professor J. W. Boyes, Chairman of the General Organizing Committee and General Secretary of the Congress. Following the official opening of the Congress and addresses of welcome, Professor Barigozzi reported on activities of the Permanent International Committee and Professor C. P. Oliver introduced Professor Sewall Wright, who then presented his Presidential Address.

OFFICIAL OPENING OF THE CONGRESS BY PROFESSOR J. W. BOYES

Mr. President, Honoured Guests, Fellow Members of the X International Congress of Genetics; Monsieur le Président, distingués invités et chers collègues du X Congrès International de Génétique:

In a chess game it is the pawn that moves first. So I stand before this distinguished platform-party of statesmen and scientists for the purpose of formally opening this tremendous Congress of Genetics. Dans une partie d'échecs c'est le pion qui fait le premier mouvement. Donc aujourd'hui, je me présente devant cette assemblée d'hommes d'état et de savants distingués dans le but d'inaugurer officiellement ce formidable Congrès de Génétique.

There are enrolled in the Congress about 1,250 Full Members, 350 Associate Members, and 200 Junior Members, plus Members of the Press. We represent over forty-five different countries. Our total budget is around $140,000, not to mention special expenses of various institutions, organizations, etc., for such items as entertainment and construction of exhibits. This represents an actual average expenditure of about ninety dollars per member, not including Junior Members.

This is the beginning of the Congress for most of you, but for those of us who have worked on its organization it represents the consummation of five years of planning, dozens of meetings, hundreds of letters, and thousands of miles of travel, not to mention the accumulation of a few gray hairs and the loss of a few pounds in weight. Pour la plupart d'entre vous, le Congrès commence, mais pour ceux d'entre nous qui ont participé à son organisation, il représente l'achèvement de cinq années de préparation, de nombreuses réunions, de centaines de lettres et de

milliers de milles de déplacement, sans mentionner l'addition de quelques cheveux gris et la perte d'un peu de poids.

A great many of our colleagues in both Canada and the United States have devoted many hours of earnest effort to the collection of Congress funds, to the distribution of funds for travel grants, to the preparation of the scientific programme, to the exhibits, to the publications, and to the arrangements for your entertainment here. In spite of all our efforts we know that there are certain faults in our arrangements and, accordingly, we earnestly request that you bear in mind that we are rank amateurs in this kind of work and forgive us for our errors. On the other hand we have succeeded far beyond our expectations in a number of ways and I have firm confidence that with your sincere co-operation we will have a most successful and memorable Congress. I should like to mention specially the exhibits prepared for you at the McGill Winter Stadium. These exhibits represent a tremendous amount of work and often considerable expense. They come to us from many countries and illustrate the grand panorama of scientific endeavour that constitutes Genetics today. Each day of the Congress from 11:00 A.M. to 9:30 P.M., for the first and only time in history, you and the public will be treated to a visual display designed to portray the Role of Genetics in the Service of Man. Je tiens à mentionner tout particulièrement l'exposition que nous avons préparée à votre intention au McGill Winter Stadium. Ils nous viennent de plusieurs pays et illustrent les merveilleuses réalisations de la génétique d'aujourd'hui.

So now on behalf of the Genetics Society of America, the Genetics Society of Canada, and the other co-sponsoring societies, it is my most pleasant duty to declare the X International Congress of Genetics officially open. Et maintenant, au nom de la Genetics Society of America, de la Société de Génétique du Canada et des autres sociétés qui nous ont prêté leurs concours, il me fait grand plaisir d'inaugurer officiellement le X Congrès International de Génétique.

Address of Welcome by the Secretary of State for Canada, the Honourable Henri Courtemanche, P.C., M.P.

Au nom du gouvernement canadien et du peuple canadien, je vous souhaite la plus cordiale des bienvenues en notre pays. C'est un honneur pour nous d'accueillir les représentants de pratiquement tous les pays du monde à l'occasion de ce X Congrès International de Génétique. Comme c'est la première fois qu'un tel événement a lieu chez nous, nous espérons que votre séjour sera des plus agréables et que notre hospitalité saura vous plaire.

In the name of the Canadian Government and of the Canadian people, it is a very pleasant duty to welcome you here today on the occasion of the X International Congress of Genetics held for the first time in Canada. It is indeed an honour to be host to scientific delegates coming from nearly all the countries in the world. I hope that your visit will be a very happy one and that you will enjoy Canadian hospitality.

D'abord, je vous demande de bien vouloir pardonner l'intrusion d'un politique dans ce cénacle d'hommes de science. A première vue, nos occupations semblent être à l'opposé, mais je crois que nous nous rencontrons en plus d'un endroit. En effet, la génétique, qui est l'étude des phénomènes de l'hérédité chez les êtres vivants est un aspect du savoir humain dont les applications concrètes ne peuvent manquer d'avoir des répercussions d'une importance capitale, tant au point de vue social qu'économique. Notre travail a donc en quelque sorte une orientation

commune: procurer à l'homme un bien-être sans cesse croissant et une sécurité réelle dans un monde meilleur.

Il faut avouer que ce n'est pas là une tâche facile. Il est vrai que l'époque que nous traversons se prête peut-être difficilement aux échanges de toutes sortes entre les pays. Mais l'étroite collaboration qu'il y a toujours eu entre les hommes de science de tous les temps nous permet de s'attendre à beaucoup de cette rencontre. L'un des buts de votre grand Congrès n'est-il pas, en effet, de resserrer les liens de compréhension et de sympathie entre les peuples? Et ce sentiment de solidarité humaine que vous êtes venu chercher ici, tout en vous proposant de définir l'état actuel de votre science, n'est-ce pas là un sentiment d'une noblesse qui se fait de plus en plus rare de nos jours?

Genetics is a field so near to man and a science of such outstanding significance in this atomic era that its future contribution to human progress and welfare is likely to be one of the most important. We have had several examples here in Canada of the essential contribution of genetics to our economy and social life. You will forgive me if, in my brief address, I confine myself to mentioning only a few phases of Canada's participation in the field of genetics. The main reason for this is my limited knowledge of the subject which, I must confess, I have acquired only since receiving the kind invitation from Mr. Boyes, your Chairman and General Secretary. No doubt the delegates of each and every country represented here today could probably say much more on their contribution, through genetics, to the welfare of humanity.

In plant science, the development of new varieties of crops has been perhaps the most spectacular achievement in Canada. We find in the *Agricultural Review* of April, 1957, this short commentary which I think is very explicit: "The plant breeding programs with cereals have revolutionized the distribution of varieties of most of our cereal crops. Marquis wheat which once made up an estimated ninety per cent of the spring wheat crop on the great plains of Canada and the United States, has been replaced completely by varieties that meet our conditions better." I have heard also that our Canadian wheat is of such outstanding quality that other countries buy it to have it mixed with their own wheat, so that the amount of protein can thus be increased.

Plant scientists here have produced new varieties of vegetables for canning suitable for the short growing seasons in most of the irrigation districts of southern Alberta. New varieties of fruits which greatly extended the cropping season were also obtained.

In the field of animal science, the use of genetic principles has given results of no less economic value. A fine example of animal-breeding research is indeed the development of the new Lacombe breed of pigs. New hybrid strains of poultry have also been well accepted by commercial poultrymen, and the cross-breeding of beef cattle to produce the famous "Cattalo" is another venture which is worth remembering.

In the social field, the burden of disease and disability will perhaps one day be kept under close control, through the efforts of specialized scientists like yourselves working in co-operation with others in different branches of science. I have heard that the Department of National Health and Welfare of the Government of Canada, in collaboration with our Dominion Bureau of Statistics and Atomic Energy of Canada, is investigating a proposal that the vital statistics system of Canada might be adapted with a view to elucidating the occurrence of genetic diseases and conditions among the Canadian population. Our well-developed, co-ordinated, and

mechanized system of recording vital statistics makes the investigation of this plan particularly promising.

The importance of genetics has long been recognized by the Canadian government. For some years, financial assistance has been provided toward fundamental and applied genetic research in Canada. For example, support from the National Health Programme has been given to McGill University for research in connection with the genetic diseases of children. Other centres of research in various parts of the country have received support from the same organization.

Vous me permettrez bien, mesdames, messieurs, de vous résumer sommairement la participation canadienne à la génétique, non pas par chauvinisme, mais bien parce que c'est là, à peu près, mes seules connaissances en ce domaine.

Vous connaissez mieux que moi notre blé Marquis et les variétés développées par la suite. Vous savez encore fort bien l'important facteur que joue le blé canadien dans les différents pays du monde, à cause de sa haute teneur en protéine. Est-il nécessaire de faire encore mention des mots Lacombe et Cattalo pour souligner la participation canadienne à la génétique?

Il vous intéresserait peut-être de savoir que le ministère canadien de la Santé nationale et du Bien-être social, de concert avec le bureau de la Statistique et la compagnie de la Couronne Atomic Energy of Canada, tente d'adopter une méthode qui jetterait quelque lumière sur les maladies héréditaires qui ont des répercussions sur la population canadienne.

L'aide financière que le gouvernement canadien a apportée au monde de la génétique n'a qu'un but, celui de travailler à améliorer le sort économico-social de ses citoyens. On objectera peut-être que nous avons suivi la vieille maxime qui veut que la charité bien ordonnée commence par soi-même. C'est tout légitime, mais il n'en reste pas moins vrai que nous n'avons jamais hésité à travailler au bien-être de l'humanité. Je ne crois pas errer en disant que nos efforts en ce domaine n'ont eu d'égal que notre travail à maintenir la paix, la paix des cœurs et des esprits, la paix des foyers et la paix des peuples.

En cette année où le monde des sciences est si actif, année de géophysique internationale, année des premières tentatives de vaincre les espaces infinis, vous avez voulu une fois de plus vous réunir pour séparer le bon grain de l'ivraie et orienter vos voiles vers les problèmes de demain, afin que vos peuples et l'humanité puissent connaître un monde meilleur.

Que vos délibérations connaissent donc tout le succès auquel elles ont droit!

Address of Welcome by the Honourable Paul Dozois, Minister of Municipal Affairs for the Province of Quebec

La province de Québec est heureuse de s'associer officiellement à l'accueil qui vous est fait, à l'occasion de ce Congrès International de Génétique tenu, pour la première fois, en terre québécoise.

Elle se réjouit en même temps des résultats qui ne manqueront pas de couronner les importants travaux scientifiques qui seront le sujet de vos discussions.

Nos valeurs humaines ne peuvent que bénéficier d'études expérimentales et de découvertes qu'un groupe aussi important de savants internationaux présenteront à ces assises scientifiques; je sais que depuis trois ans votre section canadienne, à laquelle participent nos universités, vous offre une utile collaboration.

L'exposition éducative que vous avez organisée, en marge de votre congrès, constitue, à mon sens, une attraction particulière pour tous ceux que la science

génétique intéresse puisqu'elle mettra sous leurs yeux nombre d'objets concernant la génétique en médicine, celle qui est principalement au service de l'homme.

Je suis sur que plusieurs de nos experts en science agricole tiendront à visiter cette exposition, afin d'y puiser des connaissances qui les aideront dans leur tâche.

Je remarque également, en parcourant votre programme, que vous n'avez pas oublié de fournir aux participants du congrès les moyens de se récréer dans les intermèdes d'études et de discussions, et, je souhaite qu'ils y trouvent un dérivatif agréable et reposant au cours de la semaine de délibérations.

La province a tenu à y aller de sa modeste part pour marquer l'intérêt qu'elle porte à un effort scientifique auquel se consacrent, depuis une dizaine d'années, des savants de pays européens et américains.

Nos programmes d'éducation comportent d'ailleurs des échanges internationaux dans le domaine des arts et des sciences et c'est pourquoi, tous les ans, le gouvernement que j'ai l'honneur de représenter multiplie ses bourses d'études en faveur de jeunes gens forts d'ambition et désireux de perfectionner leur savoir dans divers domaines.

De même nos grandes universités du Québec, riches de leur double culture française et anglaise, sont-elles heureuses d'inviter, sous leur toit, des savants de tous les pays dont elles apprécient le précieux apport. Les chaires qu'ils détiennent, dans ces grandes maisons d'enseignement, attirent vers leurs titulaires toute une jeunesse avide de savoir.

L'attention toute particulière que le gouvernement de la province de Québec porte à ses écoles d'agriculture tient du fait que notre province reste une province agricole, en dépit du développement industriel extraordinaire qui se produit chez nous depuis quelques années.

A quelque pays que vous apparteniez, messieurs, vous conviendrez que, chez vous comme chez nous, c'est vers la terre, mère nourricière, que l'on se tourne tout d'abord pour y puiser la vigueur nécessaire pour créer et y puiser les énergies nécessaires pour rendre nos populations heureuses.

Notre structure municipale, tout comme celle de l'éducation chez nous, est façonnée de telle sorte qu'elle répond aux besoins des localités les plus reculées, comme des centres urbains les plus modernes.

Nous nous réjouissons de ce que notre province est une sorte de siège permanent de congrès internationaux de toutes sortes.

"La belle province de Québec," un slogan bien connu dans toute l'Amérique britannique du Nord et jusqu'en Europe, reste le coin de terre hospitalier par excellence et c'est pourquoi votre choix, comme siège de votre X Congrès International, nous est particulièrement agréable.

In welcoming you officially, on behalf of the government of the province of Quebec, I wish to express our deep appreciation for the choice you have made of the seat of your X International Congress. I may assure you that you are here in a perfectly sympathetic atmosphere.

The title we enjoy of being the French province of Canada, and incidentally the largest, territorially speaking, is not conventional but essentially traditional and well expressed by our motto: "Je me souviens—I remember." Here you find, working in perfect harmony, the two great cultures which have so largely contributed to human relations in all domains.

To have a real picture of the unique character of our province, you have to get away, for a few days, from what I would call the boiling and turbulent atmosphere of the large urban centres and get closer to the quiet-living population of the rural

districts. Though we are predominant in mineral resources and produce the most electrical energy in this country, the province has remained attached to the soil and is still, therefore, a great agricultural province.

What has probably contributed largely to the development of agriculture, along with the industrial advancement, is to be found in the facilities accessible to all races and classes provided by our government, for the general welfare of the people. The co-operative system, the techniques of the soil acquired in our agricultural schools and institutes, and easy markets for his products have enabled the farmer, wherever he is established, to enjoy life and maintain the serene stability of his home.

The same may be said for the facilities enjoyed by young men who want to learn a trade, become an expert in all spheres possible; we have, at their disposal, well-trained teachers.

Going over the interesting booklet you have issued on the occasion of this international gathering, I note that our universities have been contributing to your researches and studies of genetics and I hope that they will enlarge the scope of their work in that direction.

In conclusion, permit me, Mr. President and Gentlemen, to wish you all success and fruitful results in your deliberations, on behalf of the government of the province of Quebec.

Monsieur le Président, Messieurs, j'exprime l'espoir que votre séjour à Montréal et le temps dont vous pourrez disposer pour visiter les autres parties de notre province contribueront à resserrer davantage les liens qui unissent les fervents de la science. Au nom du gouvernement de la province de Québec, je vous réitère mes meilleurs souhaits de succès pour l'issue de votre important Congrès.

Address of Welcome by Mr. C. Hugh Hanson, representing the Mayor of Montreal

The first thing which I have to say is to express to you the regrets of His Worship Sarto Fournier, our Mayor, who is not able to be present himself at the opening of this important conference, for he is out of town on a short and well-merited holiday. I consider it a privilege however that he has asked me to replace him, and in his name and that of the City of Montreal I extend to you a most cordial welcome to Montreal, the Metropolis of Canada.

I understand that this is your tenth International Congress and that it is the first time that it has been held in Canada which makes it the more gratifying that you have chosen our City. We are proud of this old city which was founded three hundred and sixteen years ago and although one hears that other cities are ambitious to pass it in size and importance, I personally, having been born here and lived here all my life, have confidence that Montreal will continue to hold her position as the metropolis of Canada.

I know that you members of this organization have come from all over the world, I also know that you have a very busy schedule, but I hope that most of you will find time to visit the points of interest in the City before your departure.

I know that this very important organization has made great contributions to the health and welfare of the world, in general. I hope that your deliberations will be most successful and as Montreal is also the second largest French-speaking city in the world, I simply end by saying "Bienvenu et nous espérons que vous reviendrez nous rendre visite bientôt."

Address by C. Barigozzi, President of the Permanent International Committee for Genetics Congresses

Mr. President, Ladies, and Gentlemen, as President of the Permanent International Committee for Genetics Congresses, which is also the Genetics Section of the International Union of Biological Sciences, I consider it a great privilege to be invited to say a few words at the opening ceremony of the X International Congress of Genetics.

I wish to recall briefly the main activities developed by the Committee during the quinquennial interval between the last International Genetics Congress (Bellagio 1953) and today.

The Committee refrained—according to tradition—from interference with the organization of the Congress which so promisingly opens today. It helped only in obtaining financial support from the International Union of Biological Sciences. The same was done for the International Genetics Symposia held in Japan in 1956, one of which (on physical and chemical approaches to problems in chromosomes) was under the auspices of the International Union of Biological Sciences. Everyone is now aware of this successful event, of which the Proceedings have already been published.

Another important achievement was the setting up of an international committee on Genetic Symbols and Nomenclature. The committee, presided over by Professor Y. Tanaka, met in Zurich in August 1957 and a report has been written, containing all the proposals of the committee. These proposals (sent to a large number of geneticists) are to be discussed at this congress so that we may achieve the international adoption of proper rules.

A third important undertaking was the preparation of new rules for electing the members of the committee. These will also be discussed and we hope that by the end of the Congress a satisfactory new system of election will be ready.

This work could not have been done without the efficient and friendly collaboration of all members, to whom I wish to convey my thanks: but the greatest help was given by our very active secretary, Professor I. M. Lerner, who deserves the gratitude of all of us.

Nothing more need be added, except a warm welcome to the forthcoming President of this committee, the chairmanship of which, according to our wise rules, I am leaving at the end of this Congress.

RECORDS OF SPECIAL CONVOCATIONS DURING THE CONGRESS

A Special Convocation of McGill University was held in the Percival Molson Memorial Stadium on Wednesday, August 20. Degrees of Doctor of Science, *honoris causa,* were conferred upon Professor Hitoshi Kihara, Professor Lionel S. Penrose, and Professor Curt Stern. The remarks of the Principal and Vice-Chancellor, Dr. F. Cyril James, are included below together with the citation of the recipients prepared by Dr. Lloyd G. Stevenson, Dean of the Faculty of Medicine, and the response of Professor Curt Stern for the recipients.

Remarks of the Principal and Vice-Chancellor

It is my privilege this morning, from this Convocation platform, to offer to each of you a warm welcome to Montreal, and especially to McGill University. I hope that the arrangements made for your comfort by the Committee headed by my colleague Professor J. W. Boyes will make your stay pleasant, and that the scientific discussions during this X International Congress of Genetics will be intellectually rewarding. When you meet again, at some other place, for the eleventh Congress, I hope that the tenth will be a pleasant memory to evoke nostalgic talk during the intervals between more serious discussion.

Genetics is a comparatively new science, but we at McGill were early indoctrinated by the enthusiasm and skill of a master. Although Leonard Huskins—whom the Genetics Society of Canada commemorates in its annual Memorial Lecture—came to McGill as Associate Professor of Botany in 1930, his enthusiasm was already focused on genetics and it was that enthusiasm which led, four years later, to the creation of our Department of Genetics of which he at once became chairman. Although more than a decade has passed since Leonard Huskins left us for the wider research opportunities at Wisconsin, I still treasure the vivid memories of his talk and readily admit that I owe to him, in large measure, such knowledge of the subject as I possess. Indeed, he almost convinced me by this evangelism that botany, zoology, and perhaps a large part of human medicine were but segments of the science of genetics and should be embraced within the scope of his department!

University administrators, I am sure you will agree, seem to suffer from a certain inelasticity of mind when confronted by suggestions of that kind, yet the work of the distinguished gentlemen who today become members of McGill University, *honoris causa*, indicates the growing significance of genetic research in many fields. No botanist can ignore the work of Professor Kihara; zoologists and entomologists read with interest the papers of Dr. Curt Stern who, like Professor Penrose, has also written much that is of professional interest to intelligent students of medicine. McGill welcomes each of these distinguished graduands, both for their personal achievements and in testimony of the growing importance of the field of knowledge with which this Congress is concerned.

If I should let my mind wander toward the 590 papers included in the volume of abstracts, or to the subject-matter of the seven symposia, I should prolong this Convocation unduly. I should, moreover, reveal the depth of my ignorance. There

Special Convocation, McGill University: *left to right*—Professor Sewall Wright, Professor L. S. Penrose, Professor Curt Stern, Professor H. Kihara, Professor F. Cyril James (Principal and Vice-Chancellor)

Special Convocation, University of Montreal: *left to right*—Dean P. Dansereau, Professor Th. Dobzhansky, Mgr le Vice-Recteur, Georges Deniger, and Professor C. H. Waddington

is, however, one other thing that I should like to say. This Congress has brought together more than fifteen hundred people from forty-five countries. That is important in itself because—whether we teach genetics, Sanskrit, or economic history—each of us has an intangible influence upon the students who come into contact with us. We are men and women, as well as specialists, and as individual citizens of our individual countries it behoves us to try to understand the thoughts and aspirations of our fellows who speak a different language and regard with loyal affection a different flag. At the end of another Congress in Philadelphia nearly two hundred years ago—the Constitutional Convention of the revolutionary American colonists—that wise old statesman Benjamin Franklin said "Gentlemen, we had better hang together, or assuredly we shall hang separately!"

These words are even more widely true today than they were then. This is not the place to analyse the dangers to peace inherent in the present international situation nor is it my function to describe the threat to civilization, and to human life itself, posed by modern weapons. I should, however, like to express the hope that, for each one of you, the personal contact with men and women from other parts of the world may be an uncovenanted benefit of this Congress, that you may return to your homes with a deeper understanding of mankind as well as a more stimulating concept of the field of Genetics.

Citations of the Recipients of Honorary Degrees Prepared by Dr. Lloyd G. Stevenson, Dean, Faculty of Medicine, McGill University

Mr. Vice-Chancellor, I have the honour to present to you in order that you may confer upon him the degree of Doctor of Science, *honoris causa*, Curt Stern. Professor Stern was born and educated in Germany but became a citizen of the United States in 1939. He received the Ph.D. degree from the University of Berlin in 1923, worked at the Kaiser Wilhelm Institute of Biology for ten years and lectured at the University for five. During the twenties and thirties he spent a number of years in the United States, chiefly as a Rockefeller Fellow at Columbia University, the University of Illinois, and the California Institute of Technology, and also as a Visiting Professor at Western Reserve University. In 1933 he was appointed Research Associate in Zoology at the University of Rochester, rising through the academic ranks to become Professor of Experimental Zoology, Chairman of the Department of Zoology, and in 1941 Chairman of the Division of Biological Sciences. Since 1947 he has been Professor of Zoology in the University of California at Berkeley.

Professor Stern has made fundamental contributions to the theory of crossing-over, to developmental genetics, to human genetics, and to radiation genetics. His proof that crossing-over is connected with exchange of chromosomal material is one of the great classical experiments. His study of the quantitative action of bobbed alleles is likewise considered to be fundamental and he has carried out some of the most thorough work ever done on position effects. Equally important is his radiation work on *Drosophila*. In human genetics his main contribution is that he has written what many consider to be the best and most important textbook in this field, to which he has also contributed as a superior teacher. His more recent work on the use of mosaics for the analysis of developmental problems is highly original and has led to great advances in the field of developmental genetics. A former President of the Genetics Society of America, Professor Stern has twice served as President of the American Society of Human Genetics. He has been a

member of the Advisory Committee on Biology and Medicine of the United States Atomic Energy Commission. One of his distinguished colleagues in genetics in another university has stated of Professor Stern that "his sweetness and humility belie his cold analytical and synthetic powers which have ranged broadly over diverse areas of heredity." His many achievements, as investigator, teacher, and author are ample warrant for the honour that I ask you now, Mr. Vice-Chancellor, to confer upon him.

Mr. Vice-Chancellor, I have the honour to present to you in order that you may confer on him the degree of Doctor of Science, *honoris causa*, Hitoshi Kihara. Professor Kihara is one of the rare scientists who has received worldwide recognition for work both theoretical and applied. He is undoubtedly one of the world's leading cytologists; at the same time he has through his own efforts and his influence promoted the application of genetics to the improvement of crop plants throughout Japan. His work on the cytogenetics of wheat is recognized by wheat breeders everywhere, East and West. He is an authority on sex chromosomes in higher plants, on the origin of common wheat, on the breeding of seedless triploid watermelons and on plant breeding by the use of artificial mutations.

Having graduated from Hokkaido Imperial University, College of Agriculture, in 1918, he continued his studies at Kyoto Imperial University, obtaining the Doctor of Science degree in 1924. Dr. Kihara then studied for two years under Professor Correns at the Kaiser Wilhelm Institute in Berlin, before his own appointment as Professor of Genetics at Kyoto Imperial University, where he remained for nearly thirty years. He is now the Director of the National Institute of Genetics, Misima. He is also the Founder and Director of the Kihara Institute for Biological Research. A former President of the Genetics Society of Japan, Professor Kihara has been awarded many honours in Japan and in the West. By virtue of his distinguished contributions to his chosen science and his statesmanship and vision as a world leader in genetics he is rightly deserving of the honour I ask you now, Mr. Vice-Chancellor, to confer upon him.

Mr. Vice-Chancellor, I have the honour to present to you in order that you may confer upon him the degree of Doctor of Science, *honoris causa*, Lionel Sharples Penrose. Educated at St. John's College, Cambridge, and at St. Thomas's Hospital Medical School, London, Professor Penrose devoted himself for eight years to medical duties and research at the Royal Eastern Counties' Institution for the mentally defective at Colchester, inaugurating his life-long interest in the problems of mental deficiency. He continued his work in psychiatric research for another six years in London, Canada, where he taught at the University of Western Ontario and in the Ontario Hospitals, of which he was Research Director. Since 1945 he has been Galton Professor of Eugenics, University College, London. A tireless investigator and prolific writer, he has published some 160 papers and several books, two of them widely recognized as classics in their kind, *The Influence of Heredity on Disease* and *The Biology of Mental Defect*. His studies have ranged over wide areas, genetic, eugenic, and chemical, and through a wide variety of diseases and deficiencies. He is an accomplished statistician, fully at home in the higher reaches of mathematics.

Professor Penrose has been accorded many honours, including the Bristowe Medal, 1929, the Buckston Browne Medal, 1933, and the Weldon Medal for Biometrics, 1950. In 1953 he became a Fellow of the Royal Society. He is now Vice-President of the Genetical Society of Great Britain. One of the most authoritative

of his American colleagues has summed up his work in very simple terms: "Professor Penrose is quite generally recognized as one of the two or three leading human geneticists." It ought to be added that in addition to his other accomplishments he has devised a number of important tests for intelligence. An expert chess player, Professor Penrose has given a practical demonstration of the importance of heredity by fathering a family of chess champions. It gives me great pleasure, Mr. Vice-Chancellor, to ask you to confer upon him a well-merited honour.

Response of Professor Curt Stern on Behalf of the Recipients of Honorary Degrees

Mr. Vice-Chancellor, in asking me to speak in acceptance of the honour which McGill University has just bestowed on us, you have placed a difficult task on me. There are good reasons why recipients of honorary degrees are usually given a silent role. Should they announce their pleasure loudly? That might appear immodest. Should they hide their gratification? That might give the impression that they make light of the gift they have been offered. Thus, silence seems a way out of embarrassment.

But if the breaking of silence poses problems for the individual recipient, these problems are multiplied if he has to express his gratitude not only for himself, but also for his colleagues. If he speaks too loudly he will not only bring censure on himself, but also displease those whose mouthpiece he is. And if he speaks too modestly, will he not seem to underrate the merits of those he represents?

Like most of the men and women who are present at this special convocation, we three have been awarded our regular doctor's degree. I doubt that we asked ourselves then whether we deserved them. We had worked hard to pass our examinations and complete our theses. While we could not claim to be truly learned men, true doctors, we were perhaps no less so than our fellow candidates.

This new degree makes us pause. Why are we thus singled out—why at least, the one who is speaking? It is true that we have tried to add to man's knowledge with our studies in genetics—but so have many others. There must have been an *embarras de richesse*, an embarrassment of riches, among geneticists from whom to choose for today's honour.

So we think of ourselves as representatives of all those assembled at this Congress and of those who could not be here. We represent three sub-populations of one great worldwide community. One of us, Hitoshi Kihara, comes from that great continent which is inhabited by more than one-half of all mankind. It is a continent with ancient civilizations which are taking on new forms. The country from which our friend comes has shown in a particularly admirable way the possibility of a synthesis of an old culture of the East with new elements adopted from the West. His countrymen have quickly grasped the methods of modern science and, beginning with Chiyomatsu Ishikawa and Toyama, many contributions to cytology and genetics were made by them.

Lionel Penrose lives close to that continent on which Mendel's discoveries were made and on which they were first taken up again. He hails from England, the country of Galton, the pioneer of studies in human heredity, and Bateson the man who named our science "Genetics."

The third one, I myself, has his home in still another part of the world. The immigrant inhabitants of his continent for centuries had depended on their countries of origin for the teaching of the wisdom and the skills of their civilization. They

now increasingly contribute their own share to the culture of the world. In the field of genetics, it is the continent of E. B. Wilson and T. H. Morgan.

Do we represent only three geographic areas? In subject-matter too, each one of us seems to stand for a different field. Plant genetics: wheat, strawberries, watermelons; human genetics: mental disease, mutations, linkage; animal genetics: *Drosophila* (and the human animal). And one more trinity: methods, and theory, and practice or ways to knowledge, knowledge for its own sake and knowledge applied in agriculture and to man himself.

Mr. Vice Chancellor! We have undertaken to ask questions concerning the honours McGill University has bestowed on us. We have tried to answer our questions. Have we interpreted correctly our roles as representatives of genetics and of geneticists at large?

Perhaps we should have accepted our pleasant fate with that attitude which does not question the wisdom of existence but humbly affirms all facts of life.

An honorary degree from McGill University carries great distinction in a general way. To geneticists a particular meaning is added. Genetics has been cultivated at McGill for a long time. Lancelot Hogben was on your faculty during some of his formative years. Leonard Huskins was first received in your Botany Department and then enabled to found an independent Department of Genetics. He assembled an active staff and trained able students. Ever since, his successor and his colleagues have added to the distinction of their department and important work in various phases of genetics has come from your campus.

We three have long been associated with McGill University in personal friendship and as colleagues in interest. We are happy from now on to be associated in a formal, special way with this institution and its traditions.

Arigato and thank you.

*

At a Special Convocation held at the University of Montreal during the evening of Thursday, August 21, degrees of Doctor of Science, *honoris causa,* were conferred upon Professor Theodosius Dobzhansky and Professor Conrad Hal Waddington. Congress members attending were welcomed by Monseigneur le Vice-Recteur, Georges Deniger. The candidates were presented by Professor Pierre Dansereau, Doyen de la Faculté des Sciences, and by Dr. Jean Beaudry, professeur agrégé à l'Institut Botanique de la Faculté des Sciences. The citations of the graduands follow.

Citations for Honorary Degrees at the University of Montreal

Monseigneur, il me fait grand plaisir de vous présenter Theodosius Dobzhansky. C'est un nom qui a autrefois paru difficile à prononcer aux Anglo-Saxons et aux Franco-Latins, mais qui aujourd'hui revient dans les discours des savants du monde entier chaque fois qu'ils discutent d'hérédité. Il revient avec une familiarité et avec une reconnaissance qui sont la marque de notre dette envers celui qui le porte. Chercheur, il a fait avancer la génétique à grands pas; professeur, il a influencé définitivement plusieurs générations d'étudiants; conférencier ou écrivain, il a diffusé largement son savoir dans des controverses sociales qui nous ont beaucoup enrichis.

Theodosius Dobzhansky est né avec le siècle à Nemirov, en Russie. Il a fait ses études à Kiev, où il a obtenu son premier poste dans l'enseignement comme assistant en Zoologie à l'Ecole Polytechnique, soit de 1921 à 1924. Il a ensuite été nommé chargé de cours en Génétique à Leningrad. Puis il vint en 1927 comme professeur agrégé de Génétique au California Institute of Technology. En 1936,

il fut promu professeur titulaire et naturalisé citoyen américain. En 1940 il était appelé à sa chaire actuelle de professeur à l'Université Columbia.

En 1926–7 il dirigeait une expédition pour l'étude des animaux domestiqués en Asie Centrale. Depuis lors, il a fait beaucoup d'autres travaux sur les terrains les plus variés, depuis la Sierra Nevada de la Californie jusqu'à la forêt ombrophile de l'Amazone.

Il est membre de plusieurs académies: l'Académie Nationale des Etats-Unis, et celles du Danemark, de la Suède et du Brésil. Il a aussi reçu la médaille D. G. Elliott de l'Académie Nationale des Etats-Unis et la médaille Kimber. Trois universités lui ont déjà décerné des doctorats *honoris causa*: Wooster, São Paulo, et Münster.

Ses publications, dont je n'ai pu vérifier le nombre exact, sont très nombreuses. Elles se classent en trois catégories: les travaux de recherche expérimentale qu'il a composés seul ou en collaboration, et qui concernent surtout les mécanismes de la transmission héréditaire; les ouvrages de synthèse où ces mêmes travaux sont intégrés dans un contexte plus large; et les articles et conférences à contenu essentiellement social, politique ou philosophique.

Cette énumération à la manière des encyclopédies ne rend aucunement compte de la vie de Theodosius Dobzhansky, une vie d'étude intense, une vie où tous les mouvements de l'âme se sont exercés avec une liberté et une force exceptionnelle. Dès maintenant les témoignages ne lui manquent pas qui attestent les qualités brillantes de son esprit, la chaleur de ses sentiments, la solidité de son influence. Il est à souhaiter qu'il prenne un jour le temps décrire son autobiographie dans le style cursif et spirituel de sa conversation quotidienne. Outre les aventures physiques et intellectuelles qu'il nous raconterait, nous y retrouverions, quelquefois entre les lignes, ces nombreuses pensées germinales dont il s'est toujours délivré avec une générosité qui font l'admiration et l'envie de ses collègues.

Il n'est pas facile de dégager, même d'une œuvre aussi mûre que celle de Theodosius Dobzhansky, les lignes maîtresses, les contributions les plus importantes, les pensées les plus définitives. Cela n'et pas facile, parce qu'il y est lui-même engagé d'une façon tellement entière encore, puisque la maturité chez lui n'a pas tari la source des inventions, et le besoin de recommencement dans des voies nouvelles où il est toujours prêt à nous conduire plutôt qu'à nous suivre.

Son œuvre publiée nous fait voir une acuité de vision extraordinaire, une capacité de mettre au foyer un problème de mécanique intracellulaire et de le contempler dans son engrenage complexe avec une patience que les répétitions ne semblent jamais lasser. Les leçons sur le gène que lui donna naguère Thomas Hunt Morgan se trouvent maintenant documentées et illustrées d'une manière véritablement exemplaire. Il faut admirer le développement de la perspective cytologique qui forme le tableau de fond dans l'œuvre de Theodosius Dobzhansky: avec les années et malgré l'élaboration graduelle d'interprétations très larges, il n'a jamais cessé, avec une humilité et une dévotion sans fléchissement, d'ajouter lui-même des petites pièces à sa mosaïque et d'en reprendre patiemment les moindres détails. Ainsi a-t-il toujours conservé un contact vital sur le plan analytique.

Ceux qui n'ont pas plongé avec lui dans les profondeurs de la microscopie nucléaire, toutefois, lui sont sans doute plus reconnaissants pour ses travaux de synthèse. Les historiens de la génétique analyseront dans le détail les diverses éditions de son *Genetics and the Origin of Species*. Ils y retraceront les pas d'une pensée alerte, aussi sensible à tous les courants de la conscience contemporaine qu'il est libre des engouements et des modes dont le milieu scientifique n'est hélas pas

exempt! Ils y trouveront, par example, une prise de contact de plus en plus ferme avec les problèmes du milieu qui conditionne la sélection naturelle. A partir d'un déterminisme cytonucléaire plutôt étroit qui était en faveur vers 1930, ils verront se dégager une compréhension plus complexe et plus large des interactions de l'hérédité et du milieu. Il n'est pas exagéré de dire que la "philosophie zoologique" de Dobzhansky est en grande partie celle de notre époque, et pour deux raisons: parce qu'il possède à fond la pensée des travailleurs contemporains et en reflète la meilleure part et aussi parce que la synthèse qu'il a su en faire à la lumière de ses propres investigations nous a tous marqués de son influence.

Non content, toutefois, de travailler dans l'isolement de son laboratoire de Columbia, de sa cabane de bois de la Sierra Nevada, de son campement dans l'Amazonie, il a voulu descendre dans l'arène et porter son message à une société qui se croit avide de valeurs scientifiques mais qui se contente trop volontiers d'avoir foi en la science sans se donner la peine de déchiffrer son contenu. Les savants qui parlent la langue du peuple sont hélas trop peu nombreux. Les savants qui parlent la langue des autres savants sont eux-mêmes assez rares. Il faut assigner à Theodosius Dobzhansky une place éminente dans la controverse qui divise le monde sur les conséquences morales et sociales de nos conceptions sur l'interaction de l'hérédité et du milieu. Dans des discours et des écrits qui visent le grand public, et où par conséquent l'hermétisme de l'essai scientifique le cède à une éloquence elle-même rigoureuse, il s'est exprimé avec une force et une autorité qui ont souvent laissé ses adversaires en panne. Il a dénoncé avec une ardeur égale tout ce qui lui paraissait violer la liberté de pensée. Il a peut-être le plus souvent élevé la voix pour défendre l'homme contre l'institution, qu'elle fut sociale, religieuse ou politique. Nombreux sont les collègues qui lui en sont reconnaissants et dont la voix s'est jointe à la sienne. Il ne leur a pas paru nécessaire de partager toutes ses vues ni même ses motifs pour réclamer avec lui le rejet des inquisitions qui menacent notre intégrité, la première des vertus professionnelles.

La Faculté des Sciences, Monseigneur, en vous demandant d'octroyer le doctorat des sciences *honoris causa* à Theodosius Dobzhansky, a obéi à un double motif. Elle a voulu profiter du Congrès International de Génétique qui se déroule actuellement dans notre ville pour honorer un savant dont l'œuvre inspire depuis longtemps l'enseignement que dispensent nos biologistes, et elle a voulu signaler à toute notre communauté la valeur exemplaire d'une œuvre qui a une très forte conscience d'elle-même et dont les répercussions atteignent les valeurs qui nous sont les plus chères.

PIERRE DANSEREAU

Monseigneur, l'Angleterre est un pays où la liberté est considérée comme le plus précieux de tous les biens et où, peut-être plus que partout ailleurs, les institutions ont graduellement et laborieusement été organisées de façon à sauvegarder jalousement cette richesse inestimable. Grâce à cette politique, l'Angleterre est devenue un foyer d'idées nouvelles et fécondes dans tous les domaines où l'homme exerce ses facultés. La philosophie, les sciences, les lettres et les arts de l'Angleterre fourmillent de noms éminents qui ont contribué à l'édification de la civilisation occidentale.

Dans le domaine de la biologie, le seul apport de Darwin suffirait à placer l'Angleterre parmi les nations d'avant-garde, mais c'est une pléiade de grands biologistes que le pays de Darwin a donnée au monde. Et pour ce qui est de la génétique, cette cadette si pleine de vie des sciences biologiques, l'Angleterre a

toujours été au premier rang, comprenant le rôle immense que peut jouer cette science pour améliorer la condition de l'homme dans un milieu aux besoins sans cesse grandissants.

Parmi l'équipe des généticiens des Iles Britanniques, Conrad Hal Waddington est un des noms les plus distingués.

Sa carrière, très variée, commença par des études en géologie et en philosophie. Tôt attiré par la biologie, il exerça d'abord son activité en embryologie. De 1933 à 1945, il fut chargé de cours en zoologie à l'université Cambridge et Fellow du Christ's College. Durant la dernière guerre, il servit son pays dans une unité de recherches de la Garde Côtière de la Royal Air Force et devint Directeur de ce service et Aviseur Scientifique auprès du Commandant-en-Chef de la Garde Côtière. Les hommes de science étaient ainsi invités pour la première fois et sur une grande échelle à formuler leurs recommandations en appliquant les méthodes et surtout l'esprit scientifique à la solution des problèmes soulevés par la poursuite des opérations militaires. Ce service connut un tel succès, que d'autres commandements créèrent des semblables et qu'il fut adopté par les Américains.

Nommé Buchanan Professor of Animal Genetics à l'Université d'Edinbourgh et Directeur honoraire du Service de Génétique animale du Conseil des Recherches agricoles de Grande Bretagne, après la guerre, il est aussi Président de la Société de Génétique de Grande-Bretagne.

Ses œuvres biologiques sont aussi nombreuses qu'importantes. En 1939, il publiait un ouvrage de génétique fort remarqué et intitulé *Introduction to Modern Genetics*. Puis, vinrent *Organizers and Genes* en 1940, *The Epigenetics of Birds* en 1952, *Principles of Embryology* en 1956 et, cette année même, *The Strategy of the Genes*. Il a en outre publié de très nombreux articles sur divers aspects de l'embryologie, de la génétique, de l'évolution et tout particulièrement de l'origine des adaptations.

Mais, ses publications reflètent une culture et des préoccupations qui dépassent les limites de sa spécialité et qui n'existent que chez quelques humanistes qui ont compris que le service de l'homme et de la pensée doit en définitive se faire par l'intégration des connaissances acquises dans toutes les sphères de l'activité humaine. Aux heures sombres de 1941, alors que tous s'inquiétaient du sort de la civilisation occidentale et cherchaient une solution à ses problèmes, le professeur Waddington signala, dans un ouvrage intitulé "The Scientific Attitude" que: "Les problèmes fondamentaux d'aujourd'hui résident beaucoup plus dans le domaine des idéals et des valeurs que dans le domaine de la technologie" et il exposa ce que l'esprit scientifique peut contribuer à l'intelligence de ces problèmes toujours actuels et à des essais de solutions. Et en 1942, toujours dans la même veine, il contribua la majeure partie d'un ouvrage écrit en collaboration et intitulé *Science and Ethics*.

C'est donc, non seulement un généticien de première valeur que l'Université de Montréal honore aujourd'hui mais aussi un penseur qui a foi dans la science et qui croit qu'elle peut et doit faire sa part pour résoudre les problèmes qui confrontent l'humanité.

Monseigneur le Vice-Recteur, j'ai le très grand honneur et le très vif plaisir de vous présenter le professeur Conrad Hal Waddington, candidat au grade de docteur ès sciences, *honoris causa*.

JEAN R. BEAUDRY

MINUTES OF THE BUSINESS MEETING AND CONCLUDING CEREMONY

THE MEETING opened at 1:40 P.M. with the President, Sewall Wright, as chairman. Professors J. W. Boyes, C. Barigozzi, G. Montalenti, I. M. Lerner, Curt Stern, and Y. Tanaka were also seated on the platform.

Professor I. M. Lerner presented the following report of the Permanent International Committee on Genetics Congresses (Genetics Section of the International Union of Biological Sciences).

Rules of Procedure

Previous Congresses have voted that our Committee become affiliated with the International Union of Biological Sciences. The many advantages of this connection carry with them one minor disadvantage: instead of operating in an informal manner we have to adopt a set of rules which are in conformity with the I.U.B.S. constitution. Accordingly, your committee has prepared the following regulations which are now presented for your approval in principle. Statements in parentheses are in the nature of explanation and do not form part of the rules.

RULES FOR ELECTION PROCEDURE TO THE GENETICS SECTION OF THE I.U.B.S.

(1) The Permanent International Committee on Genetics Congresses (the Genetics Section of the I.U.B.S.) is to be elected by the International Congresses of Genetics and hold office from the day of their election until replaced at the next Congress. (This is the current practice.)

(2) The incoming Committee will elect at its first meeting in the course of the Congress at which it is selected, a President and a Secretary. (The custom in the past has been that the past Secretary-General of the Congress becomes President and the representative of the next Congress, the Secretary. We can still follow this tradition without having it spelled out formally, although there are occasions on which for various reasons this procedure may be abandoned.)

(3) The incoming Committee shall consist of a number of members specified by the outgoing Committee on the basis of geographical grouping, taking into account the number of professional geneticists in a country and the existence of responsible professional associations. No country or region will have more than one member on the Committee. (This rule conforms to the present practice.)

(4) Outgoing members upon adoption of the distribution of seats for the next Committee will contact national or regional genetic societies and request them to submit the nominations for consideration at the next Congress. (This rule meets the I.U.B.S. requirement for the existence of national groups but does not imply the affiliation of individual societies with the I.U.B.S.)

(5) Should the national societies within a region fail to agree on a single nominee, the Congress shall select by means decided by the outgoing Committee the nominee to be presented to the Congress. (This is a rule to provide a safeguard against possible complications.)

(6) Should a region or country fail to nominate any candidate, whether by absence of any competent society or through neglect, the Committee can choose to leave the seat vacant or to request the President of the Congress to convene a nominating committee which will report to the Congress. (This rule reflects past and current practice.)

(7) The plenary sessions of the Congress may vote to change the distribution of seats proposed by the outgoing Committee. (This rule will become operative at the next Congress because no machinery for it to be invoked now could have been set up prior to the Congress itself.)

(8) Vacancies on the Committee between Congresses will be filled by the Committee itself upon nomination by the appropriate national or regional societies. (This rule requires no comment.)

(9) The first list of societies accredited to nominate representatives to the Committee is to be prepared by the group elected in 1953. Thereafter, five members of any genetic society not represented on the list can request accreditation or transfer of accreditation from another society in the same country or region. Action on such request will be taken by the incoming committee at its meeting at the Congress during which it is elected. (We already have one such request which I shall mention in the next section.)

These rules were adopted by the Congress in principle.

Distribution of Seats for the Next Committee

Twenty seats are proposed as follows, with the respective accredited societies given in parentheses:

(1) Arab countries (none)
(2) Australia and New Zealand (none)
(3) Austria and Switzerland (Swiss Society for Genetics)
(4) Belgium and Netherlands (Netherlands Genetical Society)
(5) Canada (Genetics Society of Canada)
(6) China (none)
(7) Eastern Europe (none)
(8) France (French Genetical Society)
(9) Germany (none)
(10) Great Britain (Genetical Society of Great Britain)
(11) Iberian peninsula (none)
(12) Israel (Genetic Society of Israel)
(13) Italy (Italian Genetic Society)
(14) Japan (Genetics Society of Japan; Japanese Society of Breeding; Japanese Society of Human Genetics)
(15) Scandinavia (Nordic Genetic Society)
(16) Southeastern Asia (Indian Society of Genetics and Plant Breeding)
(17) Southeastern Europe (none)
(18) South and Central America (Brazilian Society of Genetics)
(19) Union of Soviet Socialist Republics (none)
(20) United States of America (Genetics Society of America; the American Society of Human Genetics has requested accreditation which is to be considered by the incoming Committee)

Report of the Nominating Committee

The Nominating Committee appointed by President Wright (Y. Sinotô, M. Westergaard, I. M. Lerner, Chairman) presents the following list of candidates for election to the new Committee:

(1) Australia and New Zealand: Dr. O. H. Frankel, Canberra, Australia
(2) Austria and Switzerland: Professor E. Hadorn, Zurich, Switzerland
(3) Belgium and Netherlands: Professor M. J. Sirks, Groningen, Netherlands
(4) Canada: Professor J. W. Boyes, Montreal, Canada
(5) Eastern Europe: Professor K. Hruby, Praha, Czechoslovakia
(6) France: Professor B. Ephrussi, Paris, France
(7) Germany: Professor H. Stubbe, Gatersleben, Germany
(8) Great Britain: Professor C. H. Waddington, Edinburgh, Scotland
(9) Iberian peninsula: Professor A. Quintanilha, Lisbon, Portugal
(10) Israel: Professor E. Goldschmidt, Jerusalem, Israel
(11) Italy: Professor C. Barigozzi, Milan, Italy
(12) Japan: Professor D. Moriwaki, Tokyo, Japan
(13) Scandinavia: Professor H. Wexelsen, Aas, Norway
(14) Southeastern Asia: Dr. A. R. Gopal-Ayengar, Bombay, India
(15) Southeastern Europe: Professor A. Tavcar, Zagreb, Yugoslavia

(16) South and Central America: Professor F. G. Brieger, Piracicaba, Brazil
(17) Union of Soviet Socialist Republics: Professor N. P. Dubinin, Moscow, U.S.S.R.
(18) United States of America: Professor I. M. Lerner, Berkeley, California, U.S.A.

It is proposed to leave the remaining two seats, those for the Arab countries and for China, vacant for the time being.

This slate of candidates was approved by the Congress.

Place of Next Congress

Following a most warm invitation from our German colleagues, the Committee has enthusiastically voted to hold the XI International Congress of Genetics in Germany, at a place to be decided by our hosts.

Resolution on Scientific Freedom

The Permanent International Committee has adopted the following resolution introduced by Professor Møgens Westergaard. Those voting for it at the meeting were Professors Barigozzi, Brieger, Goldschmidt, Hadorn, Harland, Lerner, Sinotô, Sirks, Tavcar, Westergaard, and De Zulueta, with Professor Stubbe abstaining and Professors Ephrussi and Parthasarathy absent. The resolution is presented here not for action but merely for your information:

The Permanent International Committee on Genetics Congresses considers it to be its duty to express deep concern over the fact that a number of Soviet geneticists who had submitted abstracts of papers to the X International Congress of Genetics failed to appear in Montreal. The Committee also deeply regrets the absence of representatives at the Congress from a number of other countries. It wishes to express its deepest sympathy and send its warmest regards to all scientists who may have been prevented from attending the Congress by their governments.

The IX International Congress of Genetics, meeting in Bellagio, Italy, in 1953, passed a resolution that Genetics Congresses should not "be held in any country to which it may be expected that scientists would be refused permission to enter on grounds of race, nationality, religion, place of birth, or political associations past or present." The Permanent Committee takes this occasion to extend this policy by appealing strongly to all governments in the world to allow their scientists the right of unimpeded travel for scientific purposes, without regard of race, nationality, religion, place of birth, past or present political associations, and, in view of the experiences at the current Congress, irrespective of whether their scientific views and work are in conformity with any official governmentally shaped policies and ideology. We consider any attempt on the part of governments to interfere on political, ideological, or other grounds with a free pursuit of science and free dissemination of scientific information as a serious violation of the basic principles of research. We appeal to the learned academies and scientific societies of all countries and to the United Nations and its organizations to exert all possible influence to persuade all governments to adhere to the principles outlined here. Their violation will, no doubt, spell the end of scientific freedom and therefore also of scientific progress.

Resolution on the Organization of the Present Congress

The Committee wishes to place on record its gratification and enthusiasm for the manner of organization and functioning of the present Congress. It is obviously impossible to name individually all those who have contributed to the enormous success of these meetings. But the efforts of the general organizing committee, of the committee on exhibits, of the finance committee, the ladies' committee, the programme committee, the publications committee, and, above all, of our genial and efficient General Secretary, Professor J. W. Boyes and his staff, cannot remain unacknowledged in this report. To all of our colleagues who laboured so intensively in our cause, the Committee wishes to express its deepfelt satisfaction and thanks for their efforts. To all government bodies and agencies which have contributed finan-

cially and in other ways, and to the host institution, McGill University, the Committee also acknowledges deep appreciation.

Messages of Greetings

The Committee requests the General Secretary of the Congress to transmit in its name our warmest greetings to Professor Tschermak-Seyseneg and to Mrs. Else Goldschmidt, the widow of the President of the Ninth Congress.

Publication of Report

The Committee requests that the present report be included in the Proceedings of the X International Congress of Genetics.

Respectfully submitted, I. Michael Lerner, Secretary

Professor Y. Tanaka presented the following report of the International Committee on Genetic Symbols and Nomenclature.

In accordance with a resolution passed by the VI International Congress of Genetics in Ithaca, 1932, an International Committee meeting was held under the presidency of Professor M. J. Sirks in London in August, 1939, which passed fourteen articles of rules on the symbolization of genes. Unfortunately, they were not fully discussed and officially approved by the VII International Congress of Genetics held in Edinburgh in the same year, but they were published in *Genetica*, vol. 22, 1940. International activities stopped here and no further progress was made until a new International Committee on Genetic Symbols and Nomenclature was organized by Professor C. Barigozzi, President of the Permanent International Committee for Genetics Congresses, in 1954. It consists, after some changes, of the following members: Professor B. Ephrussi, Professor E. B. Ford, Professor E. Hadorn, Dr. A. Hagberg, Professor T. Kemp, Professor W. Landauer, Professor A. Löve, Professor H. Nachtsheim, Professor G. Pontecorvo, Professor M. M. Rhoades, and Professor Y. Tanaka, the Chairman. The Committee met in Zürich in August 1957, under the sponsorship of the International Union of Biological Sciences and the Rockefeller Foundation, and came to agreement on fourteen articles, of recommended rules. The report including those rules was printed and sent to all of you, as you know.

The Committee held its second meeting here and talked about proposed amendments, the organization of subcommittees and other matters. We have come to the conclusion that the recommended rules should not be revised and that proposals on International Subcommittees and other problems should be submitted to the coming new International Committee.

The report of our Committee was, after active discussion at the Nomenclature meeting held on August 19, 1958, unanimously approved as one of the Proceedings of this Congress.

Therefore, on behalf of the International Committee on Genetic Symbols and Nomenclature, I present our report to Professor Sewall Wright, the President of the X International Congress of Genetics, for official acknowledgment by the Congress.

The chairman noted that the printed report had been distributed and asked for discussion. There was no discussion and the report was accepted.

Professor J. W. Boyes read the following resolution received by him as Secretary General from the Genetics Society of America:

The Tenth International Congress of Genetics views with concern the increasing levels of radiation from medical, industrial and military sources to which human populations are being subjected. All such irradiation acts genetically to the detriment of this and future generations.

We call the attention of all appropriate authorities to this serious and developing situation and we urge that every attempt be made to reduce to a minimum these exposures and their ill effects by action at the international, national and individual levels.

The Congress draws attention to the valuable Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, and emphasizes the need for increased basic knowledge leading to a more comprehensive understanding and control of the damaging effects of radiation.

Professor Boyes mentioned that the delegates from Czechoslovakia withdrew an alternate resolution in favour of that presented by the Genetics Society of America. He then read the following submission from the Soviet Delegation:

The geneticists of the Soviet Union clearly understand the peril of atomic radiation for the health of man at present as well as for the future generations. Soviet geneticists understand that the greatest peril for humanity today is the testing of atomic weapons. With this in mind, they actively struggle for complete cessation of atomic tests.

It is the duty of the scientists to work and struggle for the happiness and health of humanity. Guided by this aim, Soviet scientists actively participated in the working out of the law forbidding tests of any kinds of atomic weapons which was passed by the USSR Supreme Soviet in March of this year.

The draft resolution presented to the Congress now is rather moderate. We would wish more resolute decisions to be taken by the Congress. If the Congress members decide to back this draft resolution, however, we will vote for it too.

The peril is so serious that we cannot ignore even this mild approach in the cause of obtaining the general decision on complete cessation of atomic tests so impatiently awaited by people all over the world. We ardently hope that with each new year the voice of geneticists of all the countries will sound more loudly and firmly in favour of complete elimination of any threat of atomic tests. Such is the logic of science.

We are sure that finally the peoples of the world will make their governments agree to the complete ban of both the testing and the use of atomic weapons. Such is the logic of life.

From our point of view, the basic task of geneticists is to help each man to understand the great peril of atomic radiation. At the same time each scientist should be a supporter of the cause of using atomic energy, as well as any other great scientific discoveries of the twentieth century, only for bettering the life of all people throughout the world. None of them should be used to bring misfortunes to the people.

Feeling and realizing the humane tasks, the tasks of the struggle for the happiness of humanity, we strive for the development of creative co-operation of geneticists of all nations.

After a brief discussion period the resolution presented by the Genetics Society of America was accepted on a vote of the members present.

Professor J. W. Boyes made the following brief interim report as General Secretary:

The Congress is now nearly over, each of you can judge its defects and merits for yourself, and detailed statements have no place in this meeting. So we ask that you forgive us if we have erred in some things and thank you for so many kind words of encouragement and praise.

I believe that you may be interested in a few facts about the composition and work of the Congress which I now present to you. My last count of registration gives us a total of 1,213 Active Members [later revised to 1,238] and 348 Associate Members [later revised to 350], which totals 1,561 [later revised to 1,588] in attendance at this Congress. We represent about forty-seven different countries and

perhaps you will be interested in a few figures for them. [These figures are omitted here since they are recorded elsewhere in these Proceedings.]

I think you will agree that these figures clearly demonstrate that the present Congress is the best-attended Congress of Genetics ever held. Also I should mention all the excellent work of our fund-raising organization under Drs. Frances Ryan, S. G. Smith, and R. F. Peterson. Their committees have raised around $140,000 for the Congress. The Travel Assistance Committee under Professor Mangelsdorf had the heavy responsibility of deciding who would get travel grants and they recommended 147 grants totalling over $59,000, and spread over thirty-three countries. I think these are wonderful achievements.

The very extensive work of Dr. Caspari and his Committee on the programme need not be mentioned in detail, you have all seen how well it has been done. We have a total of about 614 contributions of various kinds which has meant considerable work for Dr. Metrakos and his assistants in making local arrangements for their presentation. Drs. Beaudry and Blain have assisted us greatly where we needed help in translating into French. Dr. H. H. Smith and his associates on the Exhibits Committee have done a tremendous job on the exhibits. In the preparation of the Programme for publication Dr. Caspari has worked very closely and efficiently with me. Very extensive work has been completed by Dr. Norma F. Walker and her associates in preparing Volume II of the Proceedings, with the fine co-operation of the University of Toronto Press, on time for submission to you on the first day of this Congress. Also, Dr. Demerec has given us the valued benefit of his many years of experience.

Locally, the social activities have been the more direct responsibility of my associate, Dr. F. C. Fraser and from the kindly comments and enthusiastic reception of these events I am sure that you have appreciated them. Accommodations have been looked after by Dr. G. I. Paul and Mrs. H. Nussbaum. There has been a great deal of correspondence from my office—during the spring I was receiving over a hundred letters a day—and the fine co-operation of Mrs. Robertson, Mrs. Macmillan, and Miss Akst made it possible for us to reply swiftly to most letters. Now, of course, I should mention the work of the Ladies Committee, since Mrs. Boyes worked on this, and the arrangements for the children. Also Dr. H. Steppler made the arrangements for registration and Dr. E. R. Boothroyd for transportation and signs. They have all worked hard on the local arrangements.

I am sure that all those mentioned, and others too, merit your appreciation, and so, on behalf of the General Organizing Committee, I wish to publicly thank not only those geneticists who have worked so energetically for us, but also the large number of persons who have volunteered their services during the Congress, for their very substantial help. Also, we of the General Organizing Committee wish to assure the members of the Congress that it has been a great pleasure and honour to serve you in preparing for this great Congress of Genetics.

This report was unanimously accepted by a vote of members at the meeting.

The meeting also received the following resolutions and votes of thanks and approved them:

(*a*) A resolution presented by Professor Curt Stern: On the occasion of the fifty-year jubilee of the *Zeitschrift für Vererbungslehre*, the oldest journal devoted to Genetics, the X International Congress of Genetics sends its greetings to this international publication and expresses best wishes for its continued growth and service to Genetics.

(*b*) A vote of thanks to the organizers of the Congress presented by Professor G. Montalenti:

Mr. President, Ladies and Gentlemen, I have been asked to act as the channel through which all the participants in the X International Congress of Genetics express their feelings of appreciation and gratitude to the organizers of this meeting. I have accepted gladly this most pleasant duty.

As a member of the Executive Committee of the International Union of Biological Sciences for many years, I have witnessed several discussions and arguments as to the value of big international congresses. Nowadays, it has been said, they are too dispersive, somewhat confusing; you cannot follow everything you would like to; you see hundreds of people, but you can hardly meet a single one. Would it not be better to stop the general congresses and organize instead regional congresses or large symposia. Personally, I do not think that these criticisms are right. If a general congress is as well organized as this one, it is extremely profitable and pleasant to attend it.

I have attended by now quite a number of congresses of genetics and my recollections are quite satisfactory. Each congress has its own characteristic features, and the history of development of genetics marks at each congress, as it were, its milestones.

I will refrain from pointing out to you the major developments of our science as they come out by a mere comparison of the main topics discussed at Bellagio, only five years ago, and those which were presented at this meeting. You have heard a great number of contributions and reviews on biochemical genetics, radiation genetics, transduction, conversion, cytoplasmic inheritance, genetics and embryology, and what not.

You have seen how genetics has tackled the problem of structure of life at the undeveloped level, how genetics and biochemistry have met on fundamentally identical problems. You have, no doubt, expressed the wish that in the near future the problem of embryological differentiation may find its expression in genetic terms.

I would like to point out here two items, which are, to my mind, quite significant: *the first* is the ever growing importance of evolutionary genetics. I happened to hear, at the sixth Congress, at Ithaca, 1932, the first attempts to interpret evolution in genetic terms. Our President Sewall Wright, as you all know, was at that time an outstanding pioneer in this line of thought. Now, in the centenary of the formulation of the theory of evolution, we are glad and proud to see how much the work has progressed, how the greatest scientific generalization ever reached by biology has been confirmed by facts, and still is the most fruitful stimulus to biological investigation. The *second* fact I would like to point out is one quite outstanding feature of this Congress, namely, the demonstration of what genetics can do in the service of man.

We all are aware of how much genetics has done and how much it can do in the future for the welfare of mankind—but I think I am correct in saying that to each of us the beautiful exhibit which was so carefully and intelligently set up by the organizers of this congress was a real revelation. If we ever suffered a sort of inferiority feeling towards physics, chemistry, and other sciences which have achieved nowadays the most spectacular practical applications, I am sure that what we have seen displayed here may help us to recover from that feeling.

The documentation of the impact of genetics on human welfare, although necessarily limited, is quite impressive; therefore, in order that the wealth of demonstra-

tions and of data which have been collected here to our benefit be not dispersed and may be used for our students, for the enlightenment of public opinion, and, as the case may be, for the instruction of responsible people in our government, I would like to propose to you to carry a resolution to the effect that the material shown in the exhibits to be published in a volume, and possibly that the publication be sponsored, through the International Union of Biological Sciences, by UNESCO.

What I have said so far, interpreting, I hope, the feelings of all the participants in this Congress, is but a small fraction of what we carry in our minds at the end of this Congress. I hope, however, that it is sufficient to show how much we are indebted to all the people of good will who have prepared and organized it.

Therefore I would like to express, on behalf of all the participants, our most sincere thanks to: His Excellency the Right Honourable Vincent Massey, Governor General of Canada; McGill University; the Genetics Society of America, the Genetics Society of Canada and the other Societies who have sponsored the Congress; Mr. Henri Courtemanche, Secretary of State for the Government of Canada; Mr. Paul Dozois, Minister of Municipal Affairs of the Province of Quebec; Mr. C. Hugh Hanson, Member of the City Council, representing Mayor Fournier—whose presence at the inaugural session was a tangible token of the interest of the Government of Canada, of the Province of Quebec, of the City of Montreal in our enterprise; Dr. C. H. Goulden, Director of the Experimental Farms Service of the Canadian Department of Agriculture, who kindly acted as chairman of the public lecture in English; the Dean of the Faculty of Sciences of the McGill University which conferred the honorary degrees on Dr. Kihara, Dr. Penrose, and Dr. Stern; the Dean of the Faculty of Sciences of the University of Montreal, which conferred the honorary degrees on Dr. Dobzhansky and Dr. Waddington; the members of the General Organizing Committee, the Exhibits Committee, and the Canadian and United States Committees of Finance; the members of the Ladies' Committee, the Local Committee, the Programme Committee, the Publications Committee, and the Travel Assistance Committee; the Secretaries and Assistants. All these people have worked hard for a long time before the meeting and during the meeting to provide each one of us with a most efficient scientific meeting as well as a most pleasant stay in this beautiful city of Montreal.

Perhaps, no one, better than I and my colleague Professor Barigozzi who were involved in the organization of the Congress in Bellagio, can appreciate the tremendous effort, both financial and organizational, which is required to run in such a perfect way a huge congress like this, in which no less than sixteen hundred participants have registered, more than twice as many as in Bellagio.

I am sorry that I am not able to express personally our thanks to each one of the members of the Committees and their staff, and all collaborators, including the students and volunteer assistants, who did their best to project in the right way the slides which invariably were handed to them upside down.

I will only mention two persons, asking them to convey our thanks to their staff: namely, the chairman of the General Organizing Committee and General Secretary of the Congress, Dr. J. W. Boyes, to whose indefatigable activity and most efficient and kindly co-operative attitude the great success of this congress is essentially due; and to Mrs. Boyes, ubiquitous, always smiling and helpful to the progeny as well as to their parents, and even to old unmated males like myself.

We are leaving Montreal with the pleasant feeling of being spiritually richer than before, of having met many old friends and having made new, valuable friends, of

having discovered a new and beautiful home in which our beloved science, genetics, is being cultivated.

To all those who have made this possible, we say, from the depths of our hearts, THANK YOU.

(*c*) Professor Felix Mainx presented the following vote of thanks: My dear Professor Boyes, I would be glad if you would accept the thanks of those who received travel grants from the Committee. This grant enabled us to take part in meetings so important for all concerned with the science of genetics. We also appreciated your personal kindness and advice, and on behalf of all of us, I thank you.

(*d*) Professor Ralph Singleton introduced the following resolution: Resolved that the X International Congress of Genetics send its warmest greetings to our friend Professor William E. Castle and that a copy of this resolution be sent by our Secretary to Professor Castle.

The Business Meeting and Concluding Ceremony was officially declared closed by the chairman, President Sewall Wright, at 3:15 P.M.

OPEN LETTER TO THE PERMANENT INTERNATIONAL COMMITTEE FOR GENETICS CONGRESSES FROM THE SOVIET DELEGATION

After the end of the Congress the following letter was placed in the hands of the General Secretary with the request that it be published in the *Proceedings*. The entire letter follows:

Within the period of the work of the X International Congress of Genetics from August 20 to 27 this year, we have listened to a number of interesting papers on the actual problem of genetics. A great number of personal contacts and talks on different problems of science also undoubtedly contributed to closer bonds, mutual understanding and exchange of opinion.

The warm hospitality of Canadian scientists made our stay at the Congress pleasant and useful. We also note with satisfaction the lively interest of the Congress participants in the results of the work outlined in the papers presented by the Soviet geneticists.

But we cannot conceal one unpleasant fact. While the overwhelming majority of scientists are wholly concerned with the development of the science of heredity, certain geneticists prevent the co-operation of Soviet scientists with those of other countries.

On the day of our arrival in Montreal, the newspaper *Gazette* wrote that a number of papers by Soviet scientists allegedly had been cancelled because these papers were not in conformity with official governmental policy.

At first we looked upon this statement as a result of misunderstanding. In the course of the Congress, it turned out that a number of papers by scientists of other countries were also cancelled. Whereas this fact drew no attention, the non-arrival of some Soviet scientists was turned into a sensation.

As the Congress drew to its close, it became clear to us that some of the leading members of the previous International Permanent Committee were the original source of this interpretation of their non-arrival.

In its resolution the Permanent International Committee of the Congress especially stresses the cancellation of some Soviet papers. We have got the impression that this emphasis was not done by chance and to our regret it does not help the unity of the scientists of different countries.

Such behaviour is alien to the Soviet scientist. In our country we heartily welcome scientists from all over the world irrespective of their scientific views, race or political convictions.

In their turn Soviet scientists attend various international congresses but never before have they encountered such an experience. More than once the press mentioned the name of Professor T. Lysenko. In connection with this, we want to say that the biologists of different countries visiting the Soviet Union and wishing to meet Professor Lysenko to discuss with him the problems of genetics are always welcomed by him.

As far as international co-operation is concerned, the Soviet Union bases its attitude on the principle that each scientist has the right of free pursuit of science and the free dissemination of scientific information.

The Soviet delegation asks the Permanent Committee to publish this statement in the *Proceedings* of the X International Congress of Genetics.

PROFESSOR V. N. STOLETOV (head of the delegation)
PROFESSOR N. I. NUJDIN
PROFESSOR H. F. KUSHNER
PROFESSOR S. S. SADYKOV
PROFESSOR H. K. ENIKEEV
PROFESSOR I. E. GLOUSHTSHENKO
PROFESSOR M. M. LEBEDEV
DR. V. F. KHITRINSKY
DR. I. H. DOUKA

THE STORY OF THE X INTERNATIONAL CONGRESS OF GENETICS

J. W. Boyes, General Secretary

THE X INTERNATIONAL CONGRESS of Genetics was held at McGill University, Montreal, Canada, August 20 to 27 inclusive, 1958, under the distinguished patronage of His Excellency, the Right Honourable Vincent Massey, C.H., Governor General of Canada. The Honourable Maurice L. Duplessis, Premier of the Province of Quebec, and other eminent statesmen and noted Canadian scientists and scholars lent their patronage as members of the Honorary Committee. McGill University was host institution of the Congress which was sponsored by the Genetics Society of America with eleven other biological organizations of North America, including the recently formed Genetics Society of Canada, acting as co-sponsors. It was truly international in both organization and membership and brought into focus the great advances in genetics during the past five years.

THE INVITATION AND INITIAL STAGES

The story of the Congress really begins at the Business Meeting of the Genetics Society of America at Ithaca, New York, on September 9, 1952. At that meeting the Executive Committee recommended "that the Genetics Society of America extend an invitation to the Ninth International Genetics Congress meeting in Italy in August 1953 for the 10th International Genetics Congress to meet in the United States or Canada" and a motion to that effect by the secretary (Dr. W. R. Singleton) was approved unanimously. The writer with the support of his McGill colleagues suggested to McGill University that an invitation be extended to hold the Tenth Congress at McGill University, and the Executive Committee of the Genetics Society of America agreed to sponsor it. In March, 1953, Dr. F. Cyril James, Principal and Vice-Chancellor of McGill University, extended a formal invitation on behalf of the Board of Governors of the University through Professor C. Barigozzi, Secretary General of the IX International Congress of Genetics. The writer was instructed by McGill University to present the invitation formally at the Congress in Bellagio. This invitation was endorsed unanimously by the Permanent International Committee for Genetics Congresses and accepted at the second Business Meeting of the Ninth Congress at Bellagio in Italy. So it was decided that after the passage of twenty-six years an International Congress of Genetics would be held again in North America.

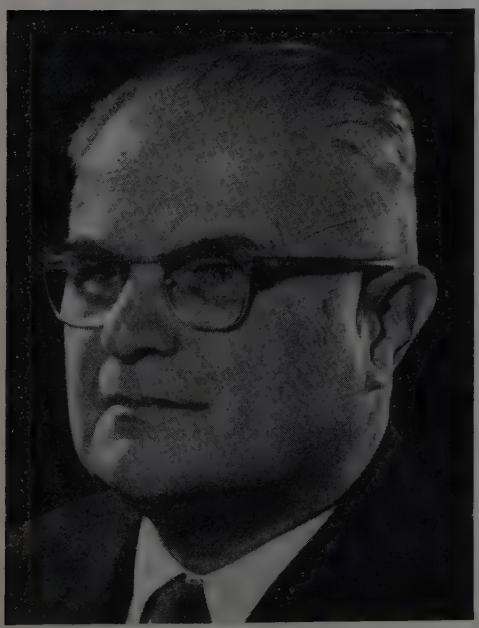
The Executive Committee of the Genetics Society of America took action soon after by inviting me as the representative of McGill University to their meeting on December 27, 1953. The Executive Committee awarded a grant of $300 to the Local Committee to help with the initial organizing expenses and at the Business Meeting the Executive Committee asked for and received authorization "to co-operate with McGill University in setting up necessary organizing committees for the Tenth Congress." The co-operative attitude of the Executive Committee was most encouraging to me and the following general plans were soon adopted:

(1) A General Organizing Committee is to be set up and will have the general responsibility for arranging the Tenth Congress, including the nature and organization of the programme,

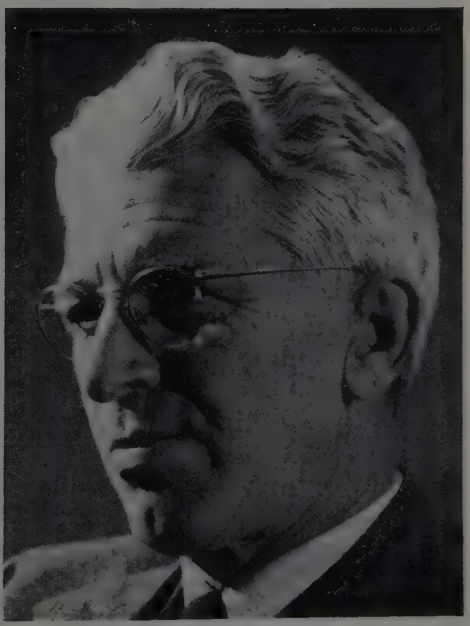
GENERAL ORGANIZING COMMITTEE

Professor J. W. Boyes, Chairman and General Secretary

Professor E. W. Caspari



Dr. M. Demerec

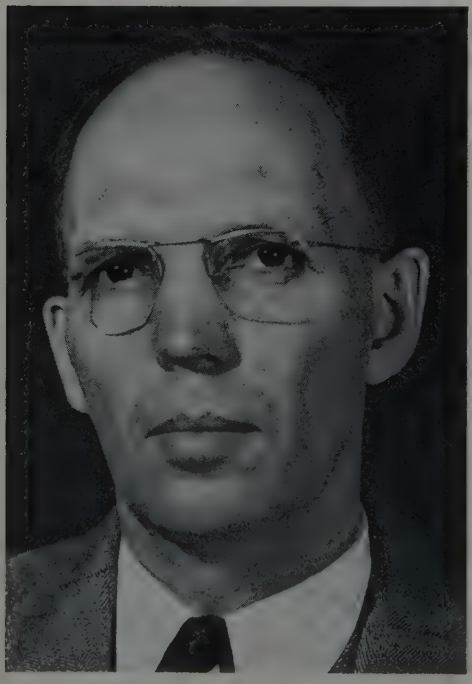


Professor P. C. Mangelsdorf

General Organizing Committee

Dr. H. B. Newcombe, *ex officio*

Professor C. P. Oliver, *ex officio*



Dr. R. F. Peterson

Professor F. J. Ryan

Dr. H. H. Smith

Dr. S. G. Smith

financial arrangements in general, the method and place of the publication of the Proceedings, and such other general matters as may come up regarding plans for the Tenth Congress.

(2) The General Organizing Committee (G.O.C.) should consist of not more than seven active members.

(3) The G.O.C. members should hold office continuously until the business of the X International Congress is completed.

(4) The G.O.C. should have authority to add to its membership if and when necessary.

(5) The Secretary of the Genetics Society of America is to be *ex-officio* a non-voting member of the G.O.C.

(6) The membership of the G.O.C. will include: (*a*) the Chairman of the Local Committee (Dr. Boyes); (*b*) the Chairman of the United States Finance Committee (Dr. F. J. Ryan was suggested); (*c*) the Chairman of the Canadian Finance Committee (Dr. S. G. Smith); (*d*) a representative from the American Society of Human Genetics (Dr. N. F. Walker); (*e*) other members to be appointed by Dr. R. E. Clausen (President of the Genetics Society of America).

(7) The G.O.C. should report at least annually (probably through its Chairman or the Secretary of the Society) to the Business Meeting of the Genetics Society of America.

(8) The G.O.C. should have authority to appoint such sub-committees as may be required; such appointed sub-committees should report directly to the G.O.C.

(9) The Local Committee will be responsible to the G.O.C. and will make all local arrangements, including the appointment of such local sub-committees as may be required. It should be responsible for implementing the general plans formulated by the G.O.C. and have considerable latitude to make decisions in order that it may be able to function efficiently.

(10) In response to the unanimous request of the Executive Committee Dr. Boyes agreed to act as Chairman of the G.O.C. since such an arrangement should result in close integration of the functions of the Local Committee and the G.O.C.

The General Organizing Committee that met first at Columbia University on May 24, 1954, had six members plus Dr. C. P. Oliver, Secretary of the Genetics Society of America, *ex-officio*. Drs. M. Demerec, Paul C. Mangelsdorf, and Francis J. Ryan represented the United States and Drs. Norma Ford Walker, S. G. Smith, and J. W. Boyes represented Canada. Dr. Ernst Caspari was asked to join the Committee in 1955 to act as Chairman of the Programme Committee, Dr. Peterson became Acting Chairman of the Canadian Finance Committee during the absence of Dr. S. G. Smith (July, 1957, to July, 1958) and was requested in October, 1957, to act as the official representative of the newly formed Genetics Society of Canada. Dr. John A. Moore represented the United States Finance Committee during the absence of Dr. Francis Ryan in Japan in the latter part of 1955 and much of 1956. Dr. H. H. Smith joined the Committee in 1957 as Chairman of the Exhibits Committee and Dr. H. Newcombe joined it as an *ex-officio* member on his election in 1956 as Secretary of the Genetics Society of America replacing Dr. C. P. Oliver.

Progress in 1954

At its first meeting, the General Organizing Committee made a number of important decisions. It was decided to suggest to the Executive Committee of the Genetics Society of America that the Society invite other societies on this continent to act as co-sponsors. The Programme Committee, with Dr. Demerec as its chairman (at his request this position was later entrusted to Dr. Ernst Caspari), the Travel Assistance Committee, with Dr. Paul Mangelsdorf as chairman, and a Publications Committee to function later, were set up. The functions of the Local Committee were discussed and it was decided that the Ladies Committee would be integrated with it; the writer agreed to act as General Secretary; the United States Finance Committee with Dr. Francis Ryan as chairman and a Canadian Finance Committee with Dr. S. G. Smith as chairman were set up; the dates of the Congress were

decided; financial requirements were carefully assessed and the handling of funds was arranged. The travel expenses ($155.79 U.S.) were paid from funds of the Genetics Society of America. Soon afterwards the General Secretary and Dr. S. G. Smith approached the Rockefeller Foundation for financial assistance for the initial organization costs and were awarded a generous grant of $3,500. On May 28 the General Secretary requested a grant of $40,000 from the Government of Canada, a request later granted in full.

The General Organizing Committee, meeting again at Columbia University on November 18, 1954, discussed many items including raising and looking after funds, expenditures, additions to the list of co-sponsors, selection of Dr. Caspari as Chairman of the Programme Committee, reports of chairmen of committees, legal responsibilities, membership of committees, lists of geneticists, and operating funds for committees.

Progress in 1955 and 1956

There were nine meetings of Congress committees in 1955. On April 23, the United States Finance Committee met and, in addition to electing Dr. John A. Moore as Acting Chairman during the absence of Dr. Ryan in Japan, decided to ask people in different regions to act as corresponding members. The Programme Committee met on May 31, 1955, at Yale University where they made general plans for the programme and also decided to select corresponding members. These were O. J. Eigsti, E. Gardner, E. L. Green, A. Hecht, I. Herskowitz, N. Horowitz, J. Lederberg, R. P. Levine, R. C. Lewontin, J. V. Neel, J. R. Preer, D. Schwartz, E. R. Sears, P. E. Stephens, C. Swanson, E. P. Volpe, and R. P. Wagner. The Local Committee had its first organizational meeting on June 10, 1955, and by this time the Congress Office had started to function on a part-time basis. Meanwhile, the Secretary of the Genetics Society of America had enlisted as co-sponsors of the Congress, the American Eugenics Society, the Agricultural Institute of Canada, the American Society of Naturalists, the American Society of Agronomy, the Society for the Study of Evolution, the American Society for Horticultural Science, the American Society of Human Genetics, the American Society for Animal Production, and the American Genetic Association. (The Genetics Society of Canada was enlisted in 1956 soon after its formation and the American Cancer Society in 1957.)

The third meeting of the General Organizing Committee was held on September 5, 1955, at East Lansing, Michigan, the day before the Annual Business Meeting of the Genetics Society of America. At the meeting the selection of the President and Vice-Presidents of the Congress was considered and there was a unanimous decision in favour of Dr. Sewall Wright as President. The members of the Travel Assistance and Local Committees were approved, we reviewed the functions of the various committees, and possible ways of getting advertising for the Congress were discussed briefly. There was some discussion of finances, of how invitations to the Congress could be extended, and of the possible impact on our Congress of the First Congress of Human Genetics to be held in 1956. At the Business Meeting the next day it was learned with appreciation that our limited funds would be increased by the $1,215 remaining in the hands of the Committee to Aid Geneticists Abroad, and by funds raised through the voluntary assessment of $2.00 each per year by members of the Genetics Society of America. The Local Committee met for the second time on October 27 for a comprehensive discussion of the organization and functions of sub-committees. On October 31, the Programme Committee met in

Montreal and discussed symposia, public meetings, invited speakers and contributed papers, corresponding members of the Committee, languages, and exhibits. In this meeting the general nature of the programme took shape clearly and it was decided to call for contributed papers in the spring of 1957 and send invitations to symposia speakers in the fall of 1957. Later on the same date, the Local Committee met with the Programme Committee to report on their work and discuss matters of common concern. Both the Travel Assistance Committee and the Canadian Finance Committee had their first meetings in Boston and Montreal respectively, on December 16, 1955. The former Committee studied its functions and the division of labour among its members; the latter devoted its efforts to a careful revision of the Congress budget from a modest $88,500 to the possible $109,000 and to consideration of methods of raising money in Canada. Thus, by the end of 1955, all the basic organizational machinery of the Congress was in operation (except for Exhibits and Publications Committees which were not needed until later).

There was a lull in preparations for the Congress in 1956. The Programme Committee met at Cold Spring Harbor, N.Y., on June 6. It decided to increase the number of sessions of contributed papers, possible topics for symposia were discussed and the general outline of the scientific programme was agreed upon. The United States Finance Committee met for the second time on November 10 and made extensive plans for canvassing foundations, government departments, industries, and private individuals in the United States in an effort to raise $25,000 for travelling expenses of overseas scientists. The Canadian Finance Committee, meeting on December 19, again considered the figure of $109,000 realistic for the budget and set an objective of raising $10,000 from provincial governments (not including Quebec) to ensure the attendance of young Canadian geneticists and of some geneticists from overseas. During this period the Congress Office in Montreal was actively assembling lists of the geneticists in all parts of the world, mailing business reply cards to those listed, writing advertising material for journals, and handling an ever increasing volume of correspondence.

Progress in 1957

On January 11, 1957, the General Organizing Committee met at Harvard University with a full agenda. By that date the finance committees had raised $11,616 and a total of $4,138 had been spent. Financial prospects were so improved that the budget was raised to $134,000. The fees were set at $15.00 for Active Members and $7.50 for Associates. Dr. Norma F. Walker agreed to act as Chairman of the Publications Committee and the University of Toronto Press was selected to publish the Proceedings. At this meeting we conceived the idea of an extensive exhibit on "The Role of Genetics in the Service of Man." Vice-Presidents were chosen (mostly as recommended by a small sub-committee of selection); it was agreed that the Honorary Committee should be chosen from Canadians; the need for an Executive Secretary was recognized; relations with other congresses were reviewed; simultaneous translation was rejected, largely because it was felt the necessary funds could be used to better purpose for travel of overseas geneticists; it was decided that a Congress crest or emblem was needed; plans for tours were reviewed; personnel were added to committees; and so on. This was a most productive meeting and set the stage for a period of activity on the part of sub-committees and the Congress Office.

The Travel Assistance Committee met at Harvard on March 25 where it discussed, among other things, chartering of a plane for Europe to Montreal flights, the arranging of an exchange visitor programme, ways of arranging lectureships for visiting geneticists, the possibility that West Coast geneticists might provide car transportation for some of our Japanese members, tours to eastern Canada and the United States, and application forms for travel grants. The Programme Committee met soon after (May 9–10) at New Haven where symposia topics and speakers were decided, the public lectures considered, and the daily schedules of activities worked out. The Ladies Committee met for the first time on May 22 to discuss general plans and methods of procedure and to outline their programme. The Local Committee met the next day and, after receiving summaries of the activities of the General Organizing Committee and of the Programme Committee plus reports of local progress, proceeded to consider appropriate tours (with Dr. Hunter representing the Travel Assistance Committee), and to assign responsibilities to members of the Committee. On May 29, Dr. Norma Ford Walker discussed details of arrangements for publication of the *Proceedings* with the members of the University of Toronto Press. In the meantime the Congress Office had received back about 1,200 of over 4,000 business reply cards sent on March 15 and later to interested geneticists, and had compiled summaries of the information obtained. The General Secretary was preparing the General Information Booklet for publication and our correspondence required daily attention.

The General Organizing Committee met on June 3 at the Carnegie Institution at Cold Spring Harbor. Plans for the programme were reported by Dr. Caspari. A system of screening applicants for grants and of approving the grants was suggested by Dr. Mangelsdorf. Dr. Ryan reported that his Committee had $30,396 on hand or assured and had plans to raise more; the Canadian Finance Committee reported receipt of over half of the $40,000 grant from the Government of Canada and was planning to approach the provinces. Plans for publication of the *Proceedings* were approved in principle, and the organization and personnel of the Local Committee were agreed upon. By this time we were assured of funds from the National Science Foundation to bring over the Japanese Exhibit, and Dr. H. H. Smith was asked to act as Chairman of the Exhibits Committee. Plans for tours were carefully examined and the most attractive ones selected. With regret it was concluded that the First International Wheat Genetics Symposium would have to be considered an affiliate of the Congress rather than a part of it. The system of handling invitations to invited speakers, individuals, institutions, and to countries for official delegations was worked out in some detail. French and English were chosen as the official languages. It was agreed that the abstracts of the contributed papers would be restricted to 300 words and that they would be published in time for the Congress as Volume II of the Proceedings. No extended publication of the contributed papers was envisaged particularly because of the high cost of printing.

The newly formed Exhibits Committee had its first meeting at Columbia University, September 10, 1957. The McGill Winter Stadium was chosen as the site and plans for construction of about a hundred units were adopted, various possible displays and the preparation of literature regarding the exhibits were considered. The General Secretary and Dr. Hunter reported on the plans for the Canadian exhibit. Dr. H. H. Smith, the Chairman, reported on his correspondence with campanies, institutions, etc., and arrangements that he was developing. Costs were discussed in general and the primary division of functions of members agreed upon. At the meeting of the Programme Committee at Columbia University on

September 25, chairmen of invited symposia and speakers at public lectures were selected; one morning session and four afternoon sessions were set aside for contributed papers and one afternoon session for demonstration papers; time was set aside for viewing the exhibits and other activities.

The General Organizing Committee met next in Montreal on October 1 with another very full agenda. Dr. Ryan advised us that there was about $40,000 in the hands of the Treasurer of the Genetics Society of America; Dr. Peterson informed us that he had asked the Government of Canada for an additional $5,000 and $1,630 had been received from provincial governments. We were thus assured of about $85,000; this was still far short of our estimated budget of $134,000, but we had prospects of obtaining more. By that time the Travel Assistance Committee had received about a hundred requests for grants.A budget of $10,000 for the Exhibits Committee was unanimously approved. We agreed that the complete manuscript of the Huskins Memorial Lecture should be included in the *Proceedings* if the Genetics Society of Canada so desired, and also that the Exhibits should be open to the public. The General Secretary reported that 229 formal invitations had been sent out to individual geneticists and 281 invitations to institutions, also that 67 invitations to countries asking for delegations were being sent out through the Department of External Affairs of Canada, and that approximately 2,000 General Information Booklets had been mailed to those who returned the card expressing interest in the Congress. Plans for the programme were reviewed and approved and arrangements for handling travel grants were clarified and completed. The Genetics Society of Canada was invited to appoint a member to represent it on the General Organizing Committee, this member to be either an existing member of the General Organizing Committee who was also a member of the Genetics Society of Canada, or an additional member of the General Organizing Committee. (The Society appointed Dr. R. F. Peterson later.) Also a design for a Congress crest produced by the General Secretary, which illustrated a chromatid exchange diagrammatically, and incorporated the interlocked ♂ and ♀ symbols the idea of Dr. J. D. Metrakos, was adopted.

Between the October meeting of the General Organizing Committee and that held on December 17 at the University of Toronto there were nine meetings of sub-committees, all attended by the General Secretary to provide integration of the various activities. The Exhibits Committee met on December 10 at Columbia where details of 80 proposed exhibits and their locations were discussed, customs regulations and procedures were studied, and questions regarding charges to exhibitors and the nature of signs, brochures, etc., were dealt with. The Canadian Finance Committee met on October 2 and again with members of the Montreal Finance Committee on December 16 at McGill University. In these meetings details of approaches to industry and provincial governments by particular individuals were worked out. At that time we were sure of $46,630 from various sources in Canada. The Travel Assistance Committee had met on October 10 and November 21 in Cambridge, and on December 16 in Montreal, and had decided upon grants for 77 applicants. They had prospects of using a total of $50,500 for travel grants to about 125 overseas geneticists. The Local Committee had meetings on October 29, November 26, and December 12 at McGill University. In these meetings detailed terms of reference were worked out for sub-committees, the registration location and procedure on arrival were planned, and reception of visitors discussed. During this fall period the Congress Office was busy preparing the advance registration, abstract and programme information forms, baggage and car stickers, the

booklet on Special Information (Publication 3), and answering many letters. We had two full-time secretaries, a half-time departmental secretary and a full-time departmental technician all working on the Congress.

It will be clear that by the end of 1957, although we were very busy on certain aspects of preparation, the Finance Committees and the Travel Assistance Committee had actually completed a great deal of their work for the Congress. At its December meeting in Toronto, the General Organizing Committee received reports of these manifold activities with thanks and approval. A sub-committee was set up, consisting of Dr. Demerec, Dr. Smith, and the General Secretary, to act on special financial problems arising out of the exhibits for which we had now a budget of $15,000. We set a deadline of March 31, 1958, for the arrival of abstracts in Dr. Walker's Toronto office and discussed other publishing plans directly with Mr. Jeanneret of the University of Toronto Press. Considerable time was spent on the selection of Vice-Presidents and candidates for honorary degrees, and on the budget.

Final Preparations, 1958

The Exhibits Committee had meetings at McGill University on February 14 and at Columbia University on May 23, 1958. Costs were worked out in more detail and individual exhibits assigned definite space. The importation of exhibits containing living materials posed special problems re bonding, duties, and brokerage fees which required careful consideration. We needed details for customs officials, and for use in the Congress Programme; so we had to prepare special letters and forms. In the final meeting such items as the language on signs, insurance, special McGill regulations regarding the Winter Stadium, personnel requirements and publicity had to be considered. For two weeks before the Congress, members of the Committee, plus three of our Japanese colleagues with a few hired assistants and volunteers, struggled to set up the exhibits in the units constructed by Clarkson Conway Associates of Montreal. Dr. Paul, the local representative of the Exhibits Committee, was very busy clearing shipments through customs. Guards, janitors, and many services were arranged by the Department of Buildings and Grounds of McGill University. Flags of many nations were borrowed from the Department of Public Works of Canada and a corral erected for the Santa Gertrudis cattle from the King Ranch. The fine co-operation and excellent efforts of this Committee produced a most impressive exhibit.

The final meeting of the Programme Committee was held at Columbia University on April 12 and 13. By this time abstracts had been received for about 510 contributed papers and 40 demonstration papers and it was now necessary to organize sessions and select chairmen for them. Dr. Caspari had done a great deal of sorting before the meeting and this saved considerable time. It was decided not to accept papers submitted without abstracts and no papers after the meeting (it was found impossible to adhere strictly to the latter decision). Several decisions regarding the public lectures and chairmen of symposia had to be made. The contributed papers were divided into sessions of fourteen or ten papers per session with eight or nine parallel sessions much as they appear in the published programme. In view of the large number of papers submitted we reluctantly had to omit time for discussion of them. The assignment of chairmen had to be left to a sub-committee which worked many hours and consulted extensively before final decisions were made. The integration of Congress social events, society meetings, and the ladies programme had to be considered. Even after this work was done, Dr. Caspari and the

General Secretary spent many hours preparing the Programme for publication. The Programme listed 596 contributed papers, 39 demonstrations, 35 invited symposia papers and 3 public lectures. The Programme Committee, and particularly its Chairman, carried a heavy load of responsibility for the success of the Congress and rightly earned the sincere thanks and admiration of its members

The eight months of 1958 that preceded the Congress were busy ones at McGill University for the Ladies Committee and the Local Committee. The ladies met each month for the first six months and the Local Committee met six times from March to June, inclusive. The main features of the Ladies Programme were worked out in January including some of the Progeny Park activities. Registration for ladies' activities, visits to museums and galleries, and the fashion show were among the topics considered in their February meeting and reported for consideration at the March 3 meeting of the Local Committee at which were also discussed details of registration, accommodation, entertainment and reception, transportation, and local programme arrangements. It was decided to arrange for a daily bulletin and we estimated costs of some activities. By March 24 the Local Committee had many more decisions to make: about bad-weather alternate arrangements for the Chicken Barbecue, food at the Marquee, local tours to the Botanical Garden and other events, getting personnel for our many services, and the heavy demand for university residence accommodation. By this date, we had received 783 advance registration forms (involving about 783 Active Members, 216 Associates, and 190 children), 525 abstracts had been received by Dr. Caspari, and we had arranged for the accommodation of 450 persons (75 per cent in University residences, 20 per cent in hotels, and 3 per cent in tent sites). Mr. James Miller was added to the Committee to keep financial records in order. The Ladies Committee planned posters of their activities, trips to art and handicraft collections, hospitals, and other events, in their April 16 meeting. On April 25 the Local Committee received reports of progress, decided to leave press releases to the General Secretary, discussed transportation costs and renting of various items of furniture, provision of personnel and costs of registrations, and other matters. By this date university residences were filled. On May 2 the Local Committee reviewed such matters as personnel requirements and details of assignments for the programme, and set up the following budget for local expenses (in Canadian dollars) of the Congress: tours, $600; registration, $1,700; ladies and reception, $2,700; programme, $800; entertainment, $1,450; transportation, $1,570; signs, $700; tickets, $150; telephones, $250; office furniture, $1,000; McGill services, $1,800; personnel, $2,000; total, $14,720. During these months the Congress Office was very busy. It sent out the booklet of Special Information together with registration and abstract forms in January and by March was receiving over a hundred letters a day. Even the addition of another full-time secretary left it hard pressed.

May and June were months of greatly increased action. Meetings of both Ladies and Local Committees involved mostly reports of activities and final decisions on hundreds of minor and a few major points. Personal contact and information were most important to members of the Local Committee in the final stages because last-minute changes in one plan might seriously affect other plans and create new problems.

The final pre-Congress meeting of the General Organizing Committee was held at the Carnegie Institution at Cold Spring Harbor. Dr. Ryan reported that his Committee had raised $59,438 for travel grants and expected about $1,000 more, not including $5,000 from the National Science Foundation for the Japanese Exhibit and $4,000 from the Rockefeller Foundation for special travel grants to

geneticists of South America. Dr. Peterson reported his Committee had raised $52,245, not including costs of the Montreal Civic Reception and an expected grant from the Province of Quebec. Dr. Mangelsdorf's Committee had acted on 223 applications for travel grants and awarded 147, involving the expenditure of $50,030. Further awards were approved and arrangements made for joint action by Dr. Mangelsdorf and the General Secretary on travel grant problems arising before or during the Congress. The Programme and plans for publications were considered and approved. Actions of the Exhibits Committee were approved with a budget of $13,500 and local arrangements were reviewed. Other items discussed included the visitor exchange programme in which Dr. Ryan had issued 104 certificates, additional Vice-Presidents, date and place of the next Congress, membership (at that time 976 Active, 297 Associates, and 215 children), tours, panel discussions, distribution of Publication No. 4, press releases, honorary degrees to be awarded, immigration and customs regulations, details of income and expenditures, galley proofs of most of Volume II of the *Proceedings*, and liability insurance. After this last meeting, except for private letters and phone calls on particular problems, the consummation of plans for the Congress was left in the hands of the Local Committee and the General Secretary.

The Congress Office was moved to the Physical Sciences Centre in April and there the items for distribution were accumulated. The General Secretary moved in next door on July 21. Soon after this it was decided to produce the special brochure for the exhibits (prepared mostly through efforts of Dr. W. F. Grant and Dr. E. R. Boothroyd, and of Dr. D. R. Sampson of Ottawa) and the final proof-reading of the Programme placed special strain on all. Up to the day before the Congress pre-registration forms were still being received. During the week before the Congress five assistants were kept busy filling the envelopes of material for members. Furniture was moved in, telephones installed, the registration hall arranged, etc. The arrival of Dr. H. H. Smith about August 10 to supervise the final erection and installation of the exhibits was a great relief. Other volunteer helpers, Dr. H. Kalter, Professors Yamashita, Tazima, Nakao, and Srb, and others, also arrived at that time or soon after and will probably never know how welcome they were. A trial run for registration was held on Monday, August 18. Then the Congress burst into a reality.

Fifty-one committee meetings preceded the Congress and there was one meeting of the General Organizing Committee during the Congress. The distribution of the pre-Congress meetings is shown in Table I. It is interesting to see how the scene shifted from year to year and to consider the tremendous amount of time and effort involved in preparation.

TABLE I

PRE-CONGRESS COMMITTEE MEETINGS

Committee	1954	1955	1956	1957	1958
General Organizing	2	1	0	4	1
Exhibits	–	–	–	2	2
Finance					
Canadian	0	1	1	2	0
U.S.	–	1	1	0	0
Ladies	–	–	–	1	6
Local	–	3	0	4	6
Programme	–	2	1	2	1
Publications	–	–	–	1	0
Travel Assistance	–	1	0	4	1

Publications Produced for the Congress

The list which follows will give a general idea of the publications produced with no mention of press releases and miscellaneous items such as certificates, post cards, stamps, and luggage stickers. All printing in Montreal was done by the Southam Printing Co. Ltd.

(1) The reply cards were printed in Montreal and 5,325 were received on February 21, 1957. On March 15, 1957, we mailed 4,330 and the remainder were sent out during the next year as requested. Of 1,555 returned: 886 wished to present papers, 638 did not wish to present papers, and the rest said they were not coming. By April 9, 1958, we had received 1,564 cards (29.4 per cent of the total sent out) and of these we had 65 per cent by May 14, 1957, 85 per cent by July 13, 1957 and 87 per cent by September 11, 1957.

(2) The *General Information Booklet* (20 pages) was printed in Montreal and 2,018 copies received June 28, 1957, were all distributed by the end of 1957.

(3) The *Special Information Booklet* (20 pages) was printed in Montreal also and of 2,531 printed, 1,639 were sent out December 23, 1957, together with sets of forms 01 (Programme: Information and Abstracts), 02 (Abstracts of Papers) and 03 (Advance Registration, Application for Membership, Accommodation and Tours); others were sent out with the forms at later dates as required. We had received 599 advance registrations by the deadline (March 15, 1958); 987 by June 3, 1958, and 1,217 by August 18, 1958, the day before registration began. Even toward the end there were ten to twenty per week. We used 2,236 sets of form 01, 2,205 of 02, and 2,974 of 03 which we tried to complete for all registrants at the Congress.

(4) Publication 4 (2 pages) was multilithed; about 4,000 copies were sent out in June, 1958.

(5) The brochure entitled *Exhibits: Genetics in the Service of Man,* (Publication 5, 10 pages) was printed in Montreal and all of the 3,020 copies received August 18, 1958, were distributed at the McGill Winter Stadium during the Congress.

(6) The *Programme* (71 pages) was printed in Montreal; 3,110 copies were received August 12, 1958, and distributed to Active and Associate Members, etc., at registration and later.

(7) The *Inaugural Session Programme* (3 pages) was printed in Montreal; 2,400 copies were received and distributed on August 20, 1958.

(8) The *Daily Bulletin* was produced by mimeographing and seven issues of 1,500 each were distributed during the Congress.

(9) Volume II of the *Proceedings* (339 pages, 596 abstracts) was printed by the University of Toronto Press under the supervision of the Publications Committee and of 2,162 copies produced 1,450 were received about August 8 for distribution to active Members at registration.

(10) Volume I of the *Proceedings* will be printed by the University of Toronto Press under the supervision of the Publications Committee and released about June, 1959, to all Active Members of the Congress.

Official Delegates to the Congress

Listed below are those for whom there are letters of designation as official delegates; apologies are extended to any who may have been missed.

Governments and Government Departments

Department of Science and Agriculture, Barbados, B.W.I., Mr. Ian Walker
Government of Denmark, Professor Tage Kemp and Professor Møgens Westergaard
East Africa High Commission, Mr. G. H. Lampkin and Mrs. K. Lampkin
Ministry of Agriculture and Lands, Kingston, Jamaica, B.W.I., Dr. T. P. Lecky
Government of the Netherlands, Mrs. Dr. A. Koopmans, Professor R. Prakken, Professor M. J. Sirks, and Dr. F. H. Sobels
Government of New Zealand, Dr. J. B. Hair
Government of Norway, Dr. Knut Mikaelsen and Professor Oeivind Nissen
Government of South Africa, Professor S. F. Oosthuizen
Department of Agriculture, South Africa, Professor F. X. Laubscher
Government of Switzerland, Professor E. Hadorn
Government of Yugoslavia, Dr. Alois Tavcar, Dr. Slavko Borojevic, and Dr. Ruzica Glavinic

Universities and Institutes

University of Bern, Professor S. Rosin
University of Birmingham, Professor D. G. Catcheside, and Professor Kenneth Mather

University of Cambridge, Dr. H. L. K. Whitehouse
University of Durham, Dr. J. L. Crosby and Dr. A. Ursula Philip
University of Edinburgh, Professor C. H. Waddington
The Hebrew University, Jerusalem, Dr. Elisabeth Goldschmidt
University of Krakow, Professor Maria Skalinska
University College, Leicester, Dr. J. R. S. Fincham
University of Liverpool, Dr. P. M. Sheppard
University of London, Professor L. S. Penrose
Istituto di Genetica Umana, Universita di Milano, Dr. A. Serra
University of Oslo, Professor O. Strömnaes
University of Paris, M. le Professeur Lamotte
The University, Sheffield, Dr. J. M. Thoday
University of Tasmania, Professor H. N. Barber
University of Trieste, Dr. Emilio Battaglia

Academies, Societies and Councils

Asociacion Uruguaya para el Progreso de la Ciencia, Montevideo, Professor F. A. Saez

Commonwealth Scientific and Industrial Research Organization, Australia, Mr. C. I. Davern, Dr. O. H. Frankel, Dr. A. S. Fraser, and Dr. F. H. W. Morley

The Eugenics Society, London, Dr. J. A. Fraser Roberts

The Science Council of Japan

Dr. Haruo Hashimoto	Dr. Seiji Matsumura	Dr. Nobunori Tanaka
Dr. Yonosuke Ikeda	Dr. Daigoro Moriwaki	Dr. Yoshimaro Tanaka
Dr. Shoei Iseki	Dr. Ujihiro Murakami	Dr. Yataro Tazima
Dr. Hitoshi Kihara	Dr. Fumihiko Ono	Dr. Yoshito Yamasaki
Dr. Hideo Kikkawa	Dr. Yosito Sinotô	Dr. Kosuke Yamashita

The Academy of Learning of the U.S.S.R.

Professor N. V. Stoletov (leader)	Dr. I. H. Douka	Dr. H. K. Enikeev
V. A. Fedoravich (secretary)	Professor I. Gloushtchenko	Mrs. T. A. Gousikova (interpreter)
Dr. V. Khitrinsky	Professor H. F. Kushner	
Professor N. I. Nujdin	Dr. S. S. Sadykov	Professor M. Lebedev

Distribution of Travel Grants

The distribution of 145 travel grants to 31 countries, totalling $57,570 (U.S.), as recommended by the Travel Assistance Committee is shown in Table II. In addition, seven grants totalling $3,600 (U.S.) were distributed to members from

TABLE II

Distribution of Travel Grants

Country	No. of grants	Total ($ U.S.)	Country	No. of grants	Total ($ U.S.)
Argentina	2	200	India	7	2,025
Australia	5	2,100	Israel	3	1,950
Austria	1	650	Italy	16	6,375
Brazil	3	1,165	Japan	21	13,295
Chile	1	700	Lebanon	1	500
China (Taiwan)	1	500	Netherlands	4	1,050
Czechoslovakia	1	650	New Zealand	2	400
Denmark	1	450	Nigeria	1	100
Egypt	1	300	Norway	2	650
Finland	3	1,000	Poland	5	1,780
France	9	3,310	Portugal	1	300
Germany	13	4,000	Spain	5	1,770
Great Britain			Sudan	1	280
England	15	3,445	Sweden	2	1,200
Scotland	11	4,450	Switzerland	2	600
Wales	1	600	Uruguay	1	750
Guatemala	1	100	Yugoslavia	2	925

South America for extra travel in the United States and Canada and $3,665 Can. from funds raised in Canada provided 26 grants to young Canadians. Thus a total of $64,835 was distributed for travel grants.

Membership Distribution in the Congress

The distribution of members according to country of origin, using the home address given, is shown in Table III. In certain cases, such as some foreign visitors and students residing in the United States, the proper home address was not available. In addition to those listed, one person, H. B. Brown, paid fees but we have no other record of him, and two libraries registered in advance in order to obtain *Proceedings*. Fifty countries were represented. There were 1,309 Active Members (of whom 71 were non-participating, that is, did not attend), 362 Associate Members (of whom 12 were non-participating), and 196 children.

The Congress Programmes

The official programme began with the Inaugural Session in the Molson Memorial Stadium, which was officially opened by Professor J. W. Boyes. Addresses of welcome were delivered for the Government of Canada, for the Province of Quebec, and for the City of Montreal. Professor Claudio Barigozzi reported as President of the Permanent International Committee for Genetics Congresses and after introduction by Professor C. P. Oliver, Professor Sewall Wright delivered his Presidential Address on "The Place of Genetics in Science" which was enthusiastically received by the audience. The Principal and Vice-Chancellor of McGill University, Dr. F. Cyril James, welcomed the Congress members to McGill University at a Special Convocation which followed the Inaugural Session and conferred honorary degrees of Doctor of Science upon Hitoshi Kihara of Japan, Lionel Penrose of England, and Curt Stern of the United States. Two other eminent members of the Congress, Theodosius Dobzansky of the United States and Conrad H. Waddington of Scotland, were awarded honorary degrees of Doctor of Science at a Special Convocation of the University of Montreal on August 21. The main scientific programme consisted of two public lectures by Professors Dobzansky and Jacob, 34 invited papers in seven symposia, 543 contributed papers in forty-three sessions, a session of 40 demonstrations, and a Panel Discussion on Teaching Genetics led by Professor Bentley Glass. A special feature of the programme was an extensive exhibit covering the entire ice area of the McGill Winter Stadium in a riot of colour. These exhibits were open daily from 11:00 A.M. to 9:20 P.M. all the days of the Congress to members and the general public. The final Business Meeting and Closing Ceremony received reports of Professor Y. Tanaka, Chairman of the International Committee on Genetic Symbols and Nomenclature, Professor I. Michael Lerner, Secretary of the Permanent International Committee for Genetics Congresses, and Professor J. W. Boyes as General Secretary of the Congress, followed by resolutions and votes of thanks.

Included among the 350 Associate Members who attended were over 300 ladies. Some ladies were attending as Active Members and, of course, many lady Associates attended the scientific meetings. Others looked after their families, explored Montreal, and attended special activities organized for them. Over 100 ladies braved

TABLE III

DISTRIBUTION OF MEMBERSHIP

Country	Active members		Associates			Country	Active members		Associates		
	Attended	N.P.*	Attended	N.P.	Children		Attended	N.P.	Attended	N.P.	Children
Argentina	4	1	–	–	–	Lebanon	1	–	–	–	–
Australia	11	–	2	–	–	Mexico	2	–	1	–	1
Austria	2	–	–	–	–	Netherlands	10	1	1	–	–
Belgium	3	1	–	–	–	New Zealand	3	–	1	–	–
Brazil	11	2	3	–	–	Nigeria	1	–	–	–	–
British West Indies	2	–	–	–	–	Norway	5	–	3	–	3
Canada	201	2	63	–	28	Pakistan	2	–	–	–	–
Chile	2	–	–	–	–	Peru	2	–	2	–	–
China (Taiwan)	1	–	–	–	–	Philippines	1	–	1	–	–
Czechoslovakia	2	–	–	–	–	Poland	6	–	–	–	–
Denmark	7	–	2	–	–	Portugal	5	1	1	–	1
Egypt	–	1	–	–	–	Puerto Rico	1	–	1	–	–
Finland	5	1	–	–	–	Roumania	–	2	–	–	–
France	13	3	1	–	–	South Africa	2	–	–	–	–
Germany	34	7	2	–	–	Spain	8	–	1	–	1
Great Britain	70	3	10	–	3	Sudan	1	–	1	–	–
Greece	–	–	1	–	–	Sweden	11	–	2	–	–
Hawaii	1	–	–	–	–	Switzerland	8	–	–	–	–
Hungary	1	2	–	–	–	Thailand	2	–	–	–	–
India	6	–	1	–	–	Turkey	1	–	–	–	2
Israel	5	3	1	–	–	United States	692	38	242	11	156
Italy	34	1	2	–	–	Uruguay	1	–	–	–	–
Japan	36	1	4	1	1	U.S.S.R.	9	1	–	–	–
Kenya	1	–	1	–	–	Venezuela	4	–	–	–	–
Korea	3	–	–	–	–	Yugoslavia	5	–	–	–	–

*N.P. = non-participating.

Dr. E. G. Heyne and family at the Registration Centre

Some Soviet members of the Congress at the Inaugural Session: *left to right*—Dr. K. K. Enikeev, Professor H. F. Kushner, Tamara Gouskova (interpreter), Professor V. Stoletov (leading delegate), Professor M. Lebedev, Professor N. I. Nujdin, Dr. V. Khitrinsky, Dr. S. Sadykov, Professor A. I. Smirnov

a cold rainy Thursday, August 21, to visit the Montreal Botanical Garden and St. Helen's Island where the steaks were good at the Hélène de Champlain Restaurant and a show of hats was interesting to the bus drivers as well as to the ladies. On the sunny Monday, August 25, over 100 ladies participated in the all-day trip into the Laurentian Mountains. In between there were visits to museums, hospitals, and art galleries and shopping trips to nearby department stores. Having children supervised at Progeny Park gave more time for relaxation, making new friends, and chatting with old ones.

Progeny Park was the centre of activities for over 200 junior visitors. Here they were free to run and play, excursions to the swimming pool were organized, and puppet shows caught their interest. Mrs. Giselle Mauer encouraged their art efforts and trained student helpers kept a constant watchful eye on the little ones. Professor Yamashita won their hearts with his Finger Magic, and a dance for teenagers developed spontaneously. That the children were not all in the Park from opening to closing every day was not the fault of the children, according to several very surprised parents. The Congress benefited also because some local parents were able to devote more time to the Congress with their own children cared for in Progeny Park.

Other Congress Activities

Social functions of the Congress were very successful. The McGill University Reception on Tuesday, August 19, arranged as a Tea and Band Concert, was a fine introduction to McGill and provided an initial opportunity to renew acquaintances and to meet other members. The Civic Reception at the Chalet on Mount Royal, overlooking downtown Montreal, was a typical example of Montreal hospitality in a lovely location, and the Chamber Music Concert that evening at Redpath Hall provided a touch of art and a relaxing interlude. The Vin d'Honneur at the University of Montreal after the Huskins Memorial Lecture on August 19, and that after the Special Convocation there on August 21, provided an opportunity for our French colleagues to extend their generous hospitality. The Chicken Barbecue at Macdonald College was attended by about 800 people, members and their children, and both weather and food were enjoyable. Banquets arranged by the Genetics Society of America, the American Society of Human Genetics and the Eugenics Society, and by the Genetics Society of Canada, were well attended and highly successful. A testimonial dinner in honour of Professor Karl Sax was also an outstanding success as was the smoker arranged by the Genetics Society of Canada on August 22. The final social event was the Smoker at the Sheraton–Mount Royal Hotel on the final evening, Tuesday, August 26. Food and beer were available in the Ballroom and there was music and dancing in the Champlain Room until long after midnight. This event provided just the right opportunity and atmosphere for a last chat and bidding adieu to friends old and new.

A decision was made early against leaving accommodation arrangements in the hands of an agency for a variety of reasons. Long before the Congress, all available accommodation in university residences was filled, except at Macdonald College. We tried to place at Macdonald only those families with cars, but could not adhere strictly to this plan. The bus service provided proved unpopular and most of those in residence there made their own arrangements to come by car. Three hundred

double rooms had been reserved at the Sheraton–Mount Royal Hotel, but these proved insufficient to take care of all those without advance registration and as a result some found hotel rooms more expensive than anticipated. The Y.M.C.A. provided a number of members with inexpensive accommodation. There was great difficulty arranging for a tent site, and fears became reality when heavy rains on Friday, August 22, deluged the site—however, the campers were moved to the Field House at McGill University's Molson Stadium, an extremely fortunate arrangement on such short notice.

A separate room equipped with typewriters and telephones was prepared for the Press. Press representatives were expected to register without charge, at which time they were given copies of the *Programme* and Volume II of the *Proceedings*. Daily press conferences were arranged by a Committee consisting of Professors E. W. Caspari, F. C. Fraser, J. R. Beaudry and the General Secretary with assistance from Mr. August Schwenk and Dr. Daphne Trasler. Several radio and television programmes featured Congress activities and persons. The local papers, and even some more distant ones such as the *New York Times*, gave the Congress ample coverage. After the Congress several television shows were organized around Congress topics; *Maclean's Magazine* reported on a panel discussion with quotations of geneticists in attendance, and it is understood that Warner Brothers are using coloured photographs of some exhibits in a film on heredity being produced for the Bell Telephone Company. The *McGill News*, published by the Graduates Society, carried some comments and the *McGill Daily*, published by the students, devoted a full page to a report of the Congress. On the whole the Congress received considerable publicity of the right sort.

Congress Tours

Tours were organized through the combined efforts of the Travel Assistance Committee and the Local Committee. The main work of this organization was delegated to a sub-committee consisting of Dr. A. W. S. Hunter in Ottawa, Dr. R. P. Levine in Boston, and Dr. W. F. Grant in Montreal, with Tobin's Travel Bureau of Montreal handling most of the business arrangements with transportation companies and hotels. As shown in Table IV, 398 persons participated in the seven tours actually held (one was cancelled). Some of those who registered and paid deposits never did go on the tour, some registered but never did pay their fees and some turned up long after the deadline for registration (indeed even up to the last minute) expecting to go. There were many problems, particularly on the longer tours: the bus broke down on one tour, there were objections to air-conditioning on another bus, the bus driver on the United States tour had never been to most of the places visited, times and customs of serving meals confused some, tipping was a trial, and many people were not properly prepared for customs and immigration requirements. Receipts were $9,586.50 and expenses $9,899.75 including $70 in early refunded deposits; there was a loss on three tours and a profit on three, with an over-all loss of $313.25. Were these tours a success? At least some assured me they were and we certainly owe real appreciation to the sub-committee that worked so hard on them.

Children at Progeny Park – *top left*, Carol Li; *lower left*, Karen Vestad, *top right*, Linda Butler; *lower right*, Darshan Pershad

Professor K. Yamashita (*left*) and Dr S. Matsumura (*right*) at the Exhibits

TABLE IV

DATA REGARDING CONGRESS TOURS

Tour	Number of people intending to go on tour by date shown					Number on tour	Number from overseas	Individual cost	Total collected	Cost	Loss (−) or profit (+)
	March 24	*	June 6	†	August 5						
Montreal Botanical Garden (2)	—	—	—	—	—	160	?	$ .50	$ 80	$ 72.00	$+ 8.00
Pre-Congress to Ottawa	14	6	15	10	10	7	6	20.00	140	175.00	− 35.00
Quebec City	25	11	32	20	44	44	35	30.00	1,320	1,234.20	+ 85.80
St. Lawrence Seaway	63	—	71	55	75	144	?	9.00	1,296	1,188.00	+108.00
Post-Congress to Ottawa	5	1	5	2	—	—	—	—	—	Cancelled	— — — — —
Niagara Falls	10	6	12	7	13	14	13	100.00	1,400	1,580.75	−180.75
Genetic laboratories, eastern United States	26	13	32	19	36	29	25	28–191.00 1–175.00	5,173	5,580.00	−407.00
						398	84%				

*Number of individuals with reservations as of March 24, 1958, who actually went on tour.
†Number of individuals with deposits paid as of June 6.

Preliminary Statement of Income

A general statement of income based on preliminary figures available on November 25, 1958 is presented below:

(*a*) Collected through the Canadian Finance Committee*		$ 53,502
Including: Government of Canada	$45,000	
Province of Quebec	3,000	
Province of Saskatchewan	1,500	
(*b*) Collected through the United States Finance Committee		68,584
Including: American Cancer Society	2,500	
Columbia University	2,000	
Genetics Society of America	6,274	
King Ranch	1,000	
National Science Foundation	26,000	
Public Health Services	10,000	
Rockefeller Foundation	9,000	
U.N.E.S.C.O. (I.U.B.S.)	3,000	
(*c*) Grants and payments to the Congress Office		5,092
Including: Rockefeller Foundation (U.S.)	3,500	
Genetics Society of America* (U.S.)	300	
(*d*) Receipts for fees (not reduced by refunds, etc.)		22,440
(*e*) Receipts for tickets		4,265
(*f*) Receipts for tours		9,587
		$163,470

*The following items are not included: (1) the Civic Reception provided by the City of Montreal (estimated cost $5,000); (2) the Reception Tea provided by McGill University (estimated cost $2,000); (3) the Provincial Reception at Quebec provided by the Quebec Department of Agriculture (estimated cost $500); (4) the Genetics Society of America also paid $156 for travel expenses of members of the General Organizing Committee attending the first meeting.

After the Congress

Perhaps for most members the Congress ended on August 27. The organizers did have a quiet little celebration party in the late afternoon of that day. But then, the signs had to come down, the furniture had to be returned, the bills paid, the exhibits returned, the telephones removed, the junk tidied up, the finances balanced, the reports written, and so on. Non-participating members had to be sent the *Programme* and Volume II of the *Proceedings*. The Publications Committee and the General Secretary had to prepare Volume I of the *Proceedings* for printing and the membership and mailing lists had to be checked and rechecked. Two secretaries were kept on for over a month after the Congress and were needed. But sooner or later all these items will be cleared away and the Congress can be considered a *fait accompli*.

In retrospect it is clear that this Congress was truly international in scope and organization. It was the largest Genetics Congress ever held with nearly 1,600 participants representing 50 different countries, not to mention some 200 children of various ages from ten countries. In organization and consummation it was a joint operation of the geneticists of Canada and of the United States. Its success is a

tribute to the interest and co-operation of geneticists from many parts of the world and clear evidence of the financial generosity of governments and government agencies in Canada and the United States, of private and public foundations, of many universities, of business and industrial concerns, and of many individuals. It was characterized by a warm feeling of friendly co-operation and by real progress in the science of genetics. President Sewall Wright during preparations for the Congress stated, "In this period of international tensions, the hope of the world rests in strengthening the bonds of understanding and sympathy among all peoples. The geneticists have an opportunity to contribute significantly to this process. We hope that the coming International Congress of Genetics will be attended by more representatives from all parts of the world than ever before and that it will be successful in strengthening our feeling of unity as well as in defining the current stage of development of our science." It can only be the sincere hope of all those who assisted that we have succeeded in these most worthy objectives.

SYMPOSIUM

THE STRUCTURE OF GENETIC MATERIAL

1. H. Fraenkel-Conrat. The Infective RNA of Tobacco Mosaic Virus (paper not published)
2. S. Benzer. The Topology and Topography of the Genetic Fine Structure (paper not published)
3. M. Demerec. Genetic Structure of the *Salmonella* Chromosome
4. J. Herbert Taylor. The Organization and Duplication of Genetic Material
5. C. Levinthall, A. Garen, and F. Rothman. Combined Genetic and Chemical Studies on a Bacterial Analysis (paper not published)

GENETIC STRUCTURE OF THE *SALMONELLA* CHROMOSOME[1]

M. Demerec[2]

DURING the past few years much progress has been made in analyzing the genetic structure of genes and chromosomes. Genetic methods of analysis have revealed the existence of smaller units, or sites, as component parts of gene loci; and it has been established that these are arranged in a linear order. Furthermore, sites have been differentiated with respect to a number of properties, such as mutability pattern. degree of phenotypic expression, and even complementary relations.

Thus, within a decade, the concept of a genome has been modified to include recognition of an infinitely larger number of genetically distinct components than was earlier anticipated; and the gene has been broken down into an aggregation of minute entities, each possessing a considerable degree of individuality. Along with these demonstrations of the diversity of the genome, however, geneticists have also acquired evidence of interrelations among its components. Although a gene can no longer be defined in terms of indivisibility by crossing over, or even in terms of non-complementation among its variants (alleles), the gene still remains an important unit of a genetic system, governing a specific biological function.

In this presentation I intend to discuss experimental results bearing on some of these considerations, with the emphasis on work done at the Department of Genetics of the Carnegie Institution of Washington by several members of my group.

GENE STRUCTURE

Physical Differentiation

Two years ago (Demerec, 1956a) I presented the data then available from our work with about four hundred auxotrophs and fermentation mutants of *Salmonella typhimurium* strains LT-2 and LT-7. In that material forty-two gene loci had been identified, and for thirty-six of them we had available for study two or more alleles (that is, mutants of independent origin). In every instance, we had detected recombination between either all the alleles or a large percentage of them, indicating that they were non-identical.

Since that time the number of mutants in our collection has tripled (it is now about twelve hundred), and at present fifty-five gene loci have been recognized, as follows:

cystine	6	proline	4
histidine	8	serine	2
leucine	2	threonine	3
isoleucine	1	tryptophan	4
isoleucine-valine	2	tyrosine	2
isoleucine-valine-leucine	1	purine	10
methionine	5	pantothenate	1
phenylalanine	2	arabinose	2

[1]Aided by a grant from the American Cancer Society.

[2]Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, New York.

The loci were identified by studies of biosynthetic blocks and by transduction experiments to determine linkage relations and complementary relations. The transduction technique was also employed to detect recombination between alleles of a gene locus. Six of the fifty-five loci investigated were represented by one mutant only, but for each of the other forty-nine we had two or more mutant alleles available. Recombination was detected between the alleles of all forty-nine loci.

Similar results have been obtained in work with *Escherichia coli*; at present we have information about six gene loci in that organism.

These data further strengthen the conclusion, reached previously, that nonidentical allelism is not a special feature of certain gene loci, but a general attribute of all.

Most of the loci studied are represented in our collection by several mutant alleles, and in some cases the number of known alleles is large, about fifty each for two loci and more than a hundred for another. As a rule, one finds very few instances of identical allelism, that is, of alleles that have assumably originated through changes at the same site of a gene locus. This fact suggests that each gene locus has a considerable number of sites at which mutations giving rise to different alleles may occur.

Phenotypic Differentiation

Two basic concepts of classical genetics, allelism and multiple allelism, postulate that a gene may exist in several forms, differing in degree of function and identifiable by the phenotypes they produce. Thus, functional differentiation within the gene has been recognized since the early days of genetic research. Once physical differentiation had been established, and methods developed for identifying the sites within a locus responsible for different allelic mutants, it became possible to determine the relative positions of individual sites and to construct maps of certain gene loci and groups of loci. In previous papers (Demerec, 1956a, b) it has been shown that *Salmonella* auxotrophs resulting from changes at different sites of a locus, although all affected primarily with respect to one nutritional requirement, may differ from one another in various other requirements. It has also been shown that the sites of alleles possessing similar requirements are not distributed at random within a locus but tend to be arranged together—the arrangement sometimes following a definite pattern.

Recently it has been shown conclusively that differentiation within a gene may even apply to complementation: in other words, certain alleles of a gene locus may complement certain others. Complementary relations between alleles have been observed in *Neurospora* (Giles, 1958; Catcheside and Overton, 1958; Case, 1958; Lacy and Bonner, 1958; Weijer, 1958; Woodward, 1958); and an impressive demonstration in *Salmonella* was provided by the analysis of histidine-requiring mutants carried out by P. E. Hartman (Hartman *et al.*, 1958).

Alleles of the same gene locus very commonly differ from one another in mutability pattern. In our work with auxotrophs, we have found characteristic differences in frequency of spontaneous reversion as well as in frequency of reversion induced by treatment with different mutagens (Demerec, 1953; Glover, 1956). A particularly striking kind of variation in mutability pattern results in what we call mutagen-stable types. These mutate spontaneously, thus demonstrating a capacity for mutation; but the degree of their mutability cannot be increased by treatment with mutagens. Z. Hartman (1956), working with serine auxotrophs, and T. Yura (1956), working with purine auxotrophs, obtained evidence that certain alleles of the same gene locus are mutagen-stable, whereas others undergo induced mutation:

that is to say, mutagen stability is a quality particular to alleles rather than to gene loci.

Studies made in our laboratory have contributed a considerable amount of data about mutagen-stable and mutagen-labile auxotrophs, some of which I shall present here. Comparative studies of mutability carried out several years ago with thirty-five auxotrophs isolated in strain B of *Escherichia coli* revealed that five of them, or 14.3 per cent, were mutagen-stable. These five were tested with ultraviolet radiation, X-rays, and several potent chemical mutagens (nitrogen mustard, manganous chloride, 1:2,3:4-diepoxybutane, and 2:4:6-tri(ethyleneimino)-1:3:5-triazine); and mutations, although they occurred spontaneously, could not be induced by any of the treatments. A considerably larger proportion of auxotrophs isolated from strains LT-2 and LT-7 of *Salmonella typhimurium* was also found to be mutagen-stable. Several of these auxotrophs were tested with all the potent mutagens we had available; but, because the preliminary results indicated that an auxotroph which did not respond to ultraviolet treatment was also not susceptible to treatment with any of the other mutagens, subsequent tests were made with ultraviolet only. Therefore, most of the findings regarding mutagen stability of LT-2 and LT-7 auxotrophs apply to ultraviolet stability in particular, although they are probably true for other mutagens as well.

In the *Salmonella* material, tests were completed with 67 *his*, 80 *pro*, and 16 *ser* auxotrophs—163 in all. In 93 of these, or 57.1 per cent, reverse mutations were not induced by ultraviolet treatment. The 163 auxotrophs tested included all those selected in strains LT-2 and LT-7 and in their derivatives from recombination experiments, except those which revert spontaneously with such high frequencies that induced reversions could not have been detected.

The analyses of inducibility of reversions in auxotrophs revealed two interesting features. They showed that in the *Salmonella* material the frequency of mutagen-stable auxotrophic mutants is very high, almost 60 per cent. They also demonstrated clearly that different genera of bacteria may differ greatly in this regard; for among the 35 *E. coli* auxotrophs tested only 14.3 per cent were mutagen-stable, whereas among the 163 *S. typhimurium* auxotrophs there were 57.1 per cent.

The five mutagen-stable *E. coli* auxotrophs are tryptophan-, histidine-, proline-, and methionine-requiring mutants. In *S. typhimurium*, mutagen-stable types have been found in all the mutant groups for which appropriate tests have been made, namely, the histidine, proline, serine, tryptophan, cystine, purine, and galactose groups. Therefore, it appears likely that mutagen-stable types are not specific to certain groups of mutants, but occur generally.

In *Salmonella*, these types have appeared frequently enough to enable us to obtain data regarding their distribution among certain groups of mutants (Table I) and

TABLE I

NUMBERS OF ULTRAVIOLET-STABLE AND ULTRAVIOLET-LABILE TYPES FOUND AMONG HISTIDINE, PROLINE, AND SERINE AUXOTROPHS DERIVED FROM STRAINS LT-2 AND LT-7 OF *Salmonella typhimurium*

Mutants	UV-labile		UV-stable		Total
	No.	%	No.	%	
Histidine	29	43.3	38	56.7	67
Proline	34	42.5	46	57.5	80
Serine	7	43.8	9	56.2	16
TOTAL	70	42.9	93	57.1	163

among alleles of certain gene loci (Tables II and III). The data of Table I show that observed frequency of mutagen-stable types was similar among three groups of mutants (histidine, proline, and serine); and the data of Tables II and III indicate that mutagen stability was found among alleles of all seven *his* and all four *pro* loci studied.

TABLE II

DISTRIBUTION OF ULTRAVIOLET-STABLE AND ULTRAVIOLET-LABILE AUXOTROPHS AMONG SEVEN *his* GENE LOCI

UV-stable	Total tested	*his* loci							
		A	B	C	D	E	F	H	?
Yes	38	4	9	5	11	4	2	1	2
No	29	4	3	6	9	2	3	1	1
TOTAL	67	8	12	11	20	6	5	2	3

TABLE III

DISTRIBUTION OF ULTRAVIOLET-STABLE AND ULTRAVIOLET-LABILE AUXOTROPHS AMONG FOUR *pro* GENE LOCI

UV-stable	Total	*pro* loci			
		A	B	C	D
Yes	45	19	16	8	2
No	31	10	17	4	0
TOTAL	76	29	33	12	2

Thus, the available experimental evidence shows that the alleles of a gene locus which can be traced to changes at single mutational sites may be either mutagen-stable or mutagen-labile, and that in *S. typhimurium* most of them are mutagen-stable. It appears probable that within a locus the sites representing mutagen-stable and mutagen-labile alleles are not grouped separately but arranged in mixed order. As the order of sites within the loci studied has not yet been positively determined, however, this question cannot be answered with certainty.

GENOMIC INTERACTIONS

Effect on Whole Genome

The results of experiments done in collaboration with T. Miyake indicate that the mutability pattern of a large number of different genes may be affected by the constitution of the genome with respect to a certain factor. In our laboratory we work mainly with two strains of *S. typhimurium*, LT-2 and LT-7, both obtained from Dr. N. Zinder. They differ strikingly in their mutability patterns. Strain LT-7 is considerably more mutable than LT-2, and auxotrophic mutants are much more easily found in LT-7 cultures. Moreover, a higher proportion of the auxotrophs isolated in LT-7 are very mutable; they revert with a frequency one hundred to one thousand times higher than that of the auxotrophs usually found in strain LT-2.

Since auxotrophs having an intermediate range of mutability are rare, classification into types showing high and low frequencies of reversion can readily be made.

Highly revertible types have been found among the alleles of all the loci studied—proof that such mutations are not limited to any one locus, and an indication that they probably are not restricted to any one group of loci (Table IV).

TABLE IV

NUMBERS OF AUXOTROPHS COLLECTED AT RANDOM IN STRAINS LT-2 AND LT-7 AND IN A PROLINE-REQUIRING MUTANT OF LT-7, AND THE PERCENTAGES OF THESE WHICH SHOW HIGH FREQUENCY OF REVERSION

Auxotroph*	LT-2		LT-7		LT-7 *pro*-47	
	Total	% high	Total	% high	Total	% high
pro	48	8.3	49	89.6	24	8.3
his	58	6.9	125	96.0	29	17.2
Others	37	8.1	34	76.5	21	0
TOTAL	143	7.7	208	91.3	74	9.5

*The auxotrophs listed as *pro* represent mutations at four proline loci; the *his* mutants represent eight histidine loci; and the "others," several leucine, isoleucine, and arabinose loci.

In preparing stock cultures of our material, we consistently inoculate with a large number of bacteria, thus improving the chances of retaining in the cultures the genetic heterogeneity derived through occurrence of mutations in them. Therefore, our stock cultures may, and usually do, carry various mutant genes in addition to the genotype each culture is intended to propagate.

It has been found that our LT-7 strain is heterogeneous with respect to presence or absence of a factor (very likely a gene) that is responsible for the characteristic mutability pattern of the strain. Among lines derived through single-colony isolation from LT-7, some exhibit the mutability pattern of LT-7 and some the mutability pattern characteristic of LT-2. In three such lines we have isolated and tested a sufficient number of auxotrophic mutants to permit identification of the mutability patterns. One of these lines originated from a single colony, which carried the *pro-47* mutation; it shows the LT-2 mutability pattern (Table IV). Another line, derived from two single-colony isolations carrying, respectively, *gal-24* and *gal-24* plus *tryD-7*, also has the LT-2 pattern; out of thirty-eight auxotrophs tested only two, or 5.3 per cent, were highly revertible. The third line has the mutability pattern of LT-7, for sixteen of twenty auxotrophs tested, or 80 per cent, were highly revertible.

Experiments are in progress to analyze the nature of the factor responsible for the more typical high-mutability pattern of strain LT–7. It appears very probable that this factor is a gene, similar to mutator genes found in *E. coli* (Treffers *et al.*, 1954; Skaar, 1956) and in several other organisms (Demerec, 1937; Ives, 1950). The evidence now available, however, indicates that the factor increases mutation rate in both directions—from wild type to auxotroph and from auxotroph back to wild type—and also that its effect is fairly general, since it has been detected in relation to at least ten gene loci. This finding provides a good illustration of close integration within a genome, where one change may affect a function—in this case, mutability pattern—of a large number of the component genes.

Interactions within Parts of a Genome

A good example of the close relations that may exist among adjacent genes of a genome is emerging from studies of the distribution of gene loci in chromosomes.

Early in research done on *Drosophila*, it was noticed that genes are not distributed randomly along a chromosome, but that some genes producing similar phenotypic effects are closely linked (Grüneberg, 1937; Komai, 1950). Such non-random distribution of functionally related genes has been observed often in our work with *Salmonella*, and, indeed, seems to be an inherent characteristic of bacteria (Demerec, 1956b). Two outstanding cases of specific gene arrangement, analyzed in *S. typhimurium*, involve genes that control related reactions, located adjacent to one another in the same order as the sequence of their biosynthetic blocks. One of these includes all the known tryptophan loci (Demerec and Z. Hartman, 1956), and the other all the known histidine loci (P. E. Hartman, 1956, and unpublished). We also have a considerable amount of evidence that the chromosome distribution of other genes may not be random, and that there is a strong tendency for genes controlling related reactions to be closely linked. A very interesting contribution to this evidence has been obtained recently by Hashimoto (see Demerec *et al.*, 1958) in his genetic studies of high streptomycin resistance and streptomycin dependence in *E. coli*.

Hashimoto succeeded in developing a technique for transducing streptomycin-sensitive bacteria to either resistance (*str-r*) or dependence (*str-d*). Reciprocal transduction tests with six *str-d* mutants showed that only one gene locus controls dependence in all of them; and further experiments with *str-r* mutants indicated that the changes responsible for *str-r* and *str-d* are either at the same gene locus or at two closely linked loci.

It is well known that *str-d* bacteria may mutate to non-dependent types, some sensitive and some resistant to streptomycin. In order to determine whether such "revertants" are due to back-mutation at the *str-d* locus or to changes in some other part of the genome, analyses were made of forty-two sensitive or slightly resistant mutants derived from *str-d*. It was found that every mutant carried an *str-d* gene; in other words, they had not originated by reversion at the *str-d* locus but by an independent mutation at another locus, which suppressed the action of *str-d*. Further tests with sixteen of these suppressor mutants showed that they are all allelic—that only one suppressor gene locus is involved. The suppressor mutations are not specific; it was observed that any one of them affected the expression of all *str-d* and *str-r* genes tested. The effect on the *str-r* phenotype is to modify it from a high degree of resistance to a low degree. Linkage relationship between the suppressor gene and *str-d* was investigated, and the data indicate close linkage between the two genes.

The results of Hashimoto's studies as a whole reveal that the genetic control of high resistance to streptomycin and dependence on streptomycin in *E. coli* resides within a short region of chromosome, which also contains a genetic mechanism—a suppressor gene—for control of the degree of resistance.

Discussion

As a result of recent discoveries, particularly the contributions arising from research with bacterial viruses and bacteria, our earlier picture of the structure of the genetic system is undergoing significant modifications. It is being both amplified and refined by the delineation of many hitherto unimagined details.

Within the past few years we have acquired new evidence of fine differentiation within genes. Here I have in mind only the biological evidence, which is entirely independent of the remarkable body of evidence regarding genic chemical structure.

We now know that genetic material comprises many mutational sites, characterized by a high degree of individuality; that a change may occur at one site without affecting the properties of adjacent sites; and that homologous sites may be interchanged between two chromosomes without change in their properties.

But at the same time it can be demonstrated that these sites are components of larger units, the genes. Changes at different sites within a gene will affect the functioning of that unit in similar, although not necessarily identical, ways. On the other hand, a change at a site belonging to another, adjacent unit will not affect the functioning of the first unit, but only that of the unit in which it occurs. This is well illustrated by the results of a genetic analysis of the histidine region of the *S. typhimurium* chromosome, made by P. E. Hartman. His data show that a change at any one of thirty-five known sites of the *hisC* locus will affect production of the enzyme imidazole-acetol-phosphate ester transaminase, whereas a change at any of twenty-five known sites of the *hisB* locus, which is adjacent to *C*, will affect production of another enzyme, imidazole-glycerol-phosphate ester dehydrase. There are indications of a sharp dividing line between the *B* and *C* loci; sites located to one side of that line control the production of one enzyme, and sites on the other side of the line do not appreciably affect that enzyme, but control the production of another.

Thus, our present evidence indicates the existence of a genetic unit, comprehending many sites, which we call a gene, although it is becoming more and more difficult to provide an exact definition of this unit. The criteria previously applied in defining the gene—that it must act as a unit in recombination and in complementation—have had to be abandoned in view of our expanding knowledge of genic properties. But lack of criteria for precise definition cannot rule out the existence of the gene as a unit. Precise definition may not be possible; or we may not as yet have sufficient knowledge for its formulation. I believe, however, that the idea of the gene as a unit of heredity is one of the most useful and valuable concepts we have, and that—for the present, at least—we should be satisfied with a broad and non-specific description, in which we regard the gene as a region of chromosome controlling a particular biological function. We should, nevertheless, always keep in mind that neither a gene nor any other genetic unit functions by itself; for the living cell, within which it acts, is a highly integrated system, where the functioning of one component is subject to and dependent on the functioning of many others. The few examples of genomic interactions discussed in this paper illustrate some of the pathways of integrated function.

REFERENCES

CASE, M. E. 1958. Linear relationships of allelic mutants at the *Pan-2* locus in *Neurospora crassa*. Proc. X Internat. Congr. Genet. *2*: 45.

CATCHESIDE, D. G., and A. OVERTON. 1958. Complementation between alleles in heterocaryons. Cold Spring Harbor Sympos. Quant. Biol. *23*: 137–140.

DEMEREC, M. 1937. Frequency of spontaneous mutations in certain stocks of *Drosophila melanogaster*. Genetics *22*: 469–478.

——— 1953. Reaction of genes of *Escherichia coli* to certain mutagens. Sympos. Soc. Exp. Biol. *7*: 43–54.

——— 1956a. A comparative study of certain gene loci in *Salmonella*. Cold Spring Harbor Sympos. Quant. Biol. *21*: 113–121.

——— 1956b. Structure and arrangement of gene loci. Cytologia, Suppl. Vol., Proc. Internat. Genet. Sympos., pp. 20–31.

DEMEREC, M., and Z. HARTMAN. 1956. Tryptophan mutants in *Salmonella typhimurium*. Genetic Studies with Bacteria, Carnegie Inst. Wash. Publ. *612*: 5–33.

DEMEREC, M.; E. L. LAHR; T. MIYAKE; I. GOLDMAN; E. BALBINDER; S. BANIC; K. HASHIMOTO; E. V. GLANVILLE; and J. D. GROSS. 1958. Bacterial genetics. Carnegie Inst. Wash. Yearb. *57*: 390–406.

GILES, N. H. 1958. Mutations at specific loci in *Neurospora*. Proc. X Internat. Congr. Genet. *1*: 261–279.

GLOVER, S. W. 1956. A comparative study of induced reversions in *Escherichia coli*. Genetic Studies with Bacteria, Carnegie Inst. Wash. Publ. *612*: 121–136.

GRÜNEBERG, H. 1937. Gene doublets as evidence for adjacent small duplications in *Drosophila*. Nature *140*: 932.

HARTMAN, P. E. 1956. Linked loci in the control of consecutive steps in the primary pathway of histidine synthesis in *Salmonella typhimurium*. Genetic Studies with Bacteria, Carnegie Inst. Wash. Publ. *612*: 35–61.

HARTMAN, P. E.; Z. HARTMAN; D. SERMAN; and J. C. LOPER. 1958. Genetic complementarity in histidineless *Salmonella typhimurium*. Proc. X Internat. Congr. Genet. *2*: 115–116.

HARTMAN, Z. 1956. Induced mutability in *Salmonella typhimurium*. Genetic Studies with Bacteria, Carnegie Inst. Wash. Publ. *612*: 107–120.

IVES, P. T. 1950. The importance of mutation rate genes in evolution. Evolution *4*: 236–252.

KOMAI, T. 1950. Semi-allelic genes. Am. Natur. *84*: 381–392.

LACY, A. M., and D. M. BONNER. 1958. Complementarity between alleles at the *Td* locus in *Neurospora crassa*. Proc. X Internat. Congr. Genet. *2*: 157.

SKAAR, P. D. 1956. A binary mutability system in *Escherichia coli*. Proc. Nat. Acad. Sc. U.S. *42*: 245–249.

TREFFERS, H. P., V. SPINELLI, and N. O. BELSER. 1954. A factor (or mutator gene) influencing mutation rates in *Escherichia coli*. Proc. Nat. Acad. Sc. U.S. *40*: 1064–1071.

WEIJER, J. 1958. Evidence for a complex structure of the *td*-locus in *Neurospora crassa*. Proc. X Internat. Congr. Genet. *2*: 310.

WOODWARD, D. O. 1958. Heterocaryon complementation between adenylosuccinaseless mutants of *Neurospora crassa*. Proc. X Internat. Congr. Genet. *2*: 321.

YURA, T. 1956. Evidence of nonidentical alleles in purine-requiring mutants of *Salmonella typhimurium*. Genetic Studies with Bacteria, Carnegie Inst. Wash. Publ. *612*: 63–75.

THE ORGANIZATION AND DUPLICATION OF GENETIC MATERIAL[1]

J. Herbert Taylor[2]

One of the well-known achievements of cytogenetics is the discovery that genetic material is organized into discrete units, the chromosomes. Although the number and size of these units vary greatly among the many species of organisms, their individuality and characteristic structure are among the most permanent features of a species. When genetic analysis is extended to micro-organisms and viruses where cytological evidence is still incomplete, the same principle applies. However, in spite of their individuality and characteristic structure, chromosomes are capable of breaking and rejoining with each other, not only at homologous loci, but presumably at any locus. Therefore, they must have some common structural feature. Whether this cytological similarity extends to the micro-organisms and viruses remains uncertain, but genetic analysis of recombination suggests that they too share some common structural features.

Since their discovery chromosomes have been recognized as rod-shaped bodies that typically appear at division stages. For more than twenty-five years, these rod-shaped bodies have been generally recognized to be formed by a helical coil of a much longer filament. The structure and the three-dimensional shape of this filament still remain in doubt in spite of the fact that it is frequently described as a string of chromomeres or a beaded filament. The cytologist's concept of this filament has often been that of a bundle of fibrils which are subdivided into the two chromatids at prophase. Evidence has been brought forward at various times to indicate that the chromatids are further subdivided into half-chromatids and sometimes into quarter-chromatids (see Ris, 1957, for a detailed presentation of this concept). On the other hand, the well-known fact that crossing-over regularly occurs at the chromatid level would tend to discount the validity of any functional subdivision beyond the level of the chromatid.

New Approaches in Chromosome Analysis

In view of this perplexing situation, which has failed to yield to the methods available to cytogeneticists during the last twenty-five or thirty years, new methods of analysis are most welcome. There are several of these which I believe will give us a much clearer picture of the organization of genetic material within the next decade. The first break came with the discovery that DNA (deoxyribonucleic acid) was genetic material (Avery *et al.*, 1944). It is not necessary to recount for geneticists the developments that followed in that field. As a result of the renewed interest in DNA, biochemists and crystallographers have given us a picture of its structure.

[1]The original experiments reported are part of an investigation supported by grants from the Atomic Energy Commission, Contract AT(30–1) 1304, and the Higgins Fund of Columbia University. Technical assistance of Miss Dorothy Pfeffer is gratefully acknowledged.

[2]Department of Botany, Columbia University.

With this as a basis for a more refined type of genetic analysis (discussed by Benzer, 1957, and by Benzer, Demerec, and Levinthal in this symposium) and a more critical and careful analysis of aberrant genetic recombinations in tetrads, we have the beginnings of a new understanding of genetic fine structure that may extend from the phages to higher organisms. At the cytological level information from biochemistry and biophysics has provided us with a powerful new tool. The first step was the discovery that thymidine is a highly selective precursor for DNA (Reichard and Estborn, 1951; and Friedkin *et al.*, 1956). The next step was the demonstration that tritium would give excellent resolution in autoradiography because most of its soft radiation is stopped in the first micron of photographic emulsion applied to an object (Fitzgerald *et al.*, 1951). It remained then to label thymidine with tritium in a stable position at high specific activity and use it for tracing the behavior of DNA during chromosome duplication (Taylor, Woods, and Hughes, 1957; Taylor, 1958a, 1958b, 1958c). These experiments gave us for the first time a clear picture of the number of functional units or strands of a chromosome and the mode of segregation of these strands during duplication and the subsequent divisions.

Autoradiographic Studies with Thymidine-H^3

Chromosomes can be labelled with thymidine-H^3 only during the interphase when they are duplicating. Several hours are then required before the labeled chromosomes appear in division figures. In root cells of *Bellevalia* this period is about six to eight hours at 25° C. When they do appear, each of the two chromatids is labeled (Fig. 1) and furthermore, is equally labeled within the limits of error of the autoradiographic method (Taylor, 1958a).

Colchicine is a convenient tool for increasing the frequency with which the chromosomes may be separated and flattened for autoradiography. The spindle is destroyed and the two chromatids often remain united at the centromere for a longer period. However, they frequently spread far enough apart in squashes to allow good resolution of the label in each.

The equal distribution of the label at the first division after a single duplication in thymidine-H^3 tells us very little by itself. Each chromosome either could have divided and built a new half, or all the DNA could have been destroyed and replaced by new DNA. But at the next division, after one duplication free of the labeled precursors, the autoradiographs yielded the significant answer. The labeled chromosomes regularly produced one labeled chromatid and one completely free of label (Fig. 2). Since these labeled anaphase chromosomes, which had duplicated once in thymidine-H^3, conserved this DNA and passed it on to only one of their daughter chromosomes, the original unlabeled DNA must likewise have been conserved and passed on to the other daughter. Therefore, the chromosomes must have been composed of two sub-units of DNA. One was the original unlabeled unit inherited during duplication and the other was a new unit built when duplication occurred in thymidine-H^3.

Chromosomes, then, are composed of two and only two functional sub-units of DNA. These are conserved at each duplication with two new sub-units built, one for each chromatid. A chromatid at each division is one-half old and one-half new.

The second division was first studied by leaving the labeled cells in colchicine from just before the first division, that is, after eight hours in thymidine-H^3, until they reached a second division. Since the chromosomes from the first division were held in one cell under the action of colchicine, the second divisions could be recog-

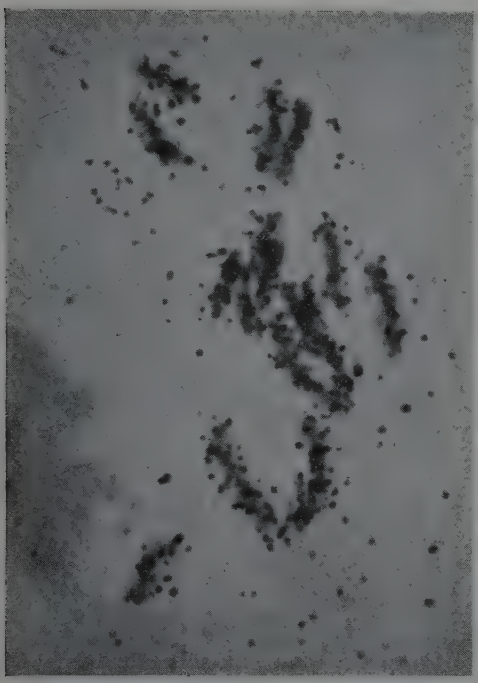


FIGURE 1. Autoradiograph of the chromosomes in a root-tip cell of *Bellevalia* after one duplication in tritium-labeled thymidine; colchicine-blocked division (after Taylor 1958b) (×1650).

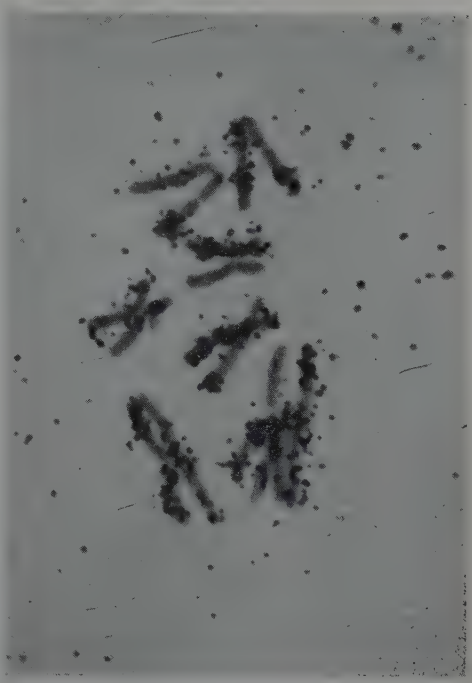


FIGURE 2. Autoradiograph of a similar cell fixed after one duplication in tritium-labeled thymidine and one duplication in a medium free of labeled precursors; colchicine-blocked metaphase (after Taylor, 1958a) (×1650).

nized because the cells regularly contained double the usual number of chromosomes. In later experiments roots were grown for eight hours in thymidine-H^3, washed, and transferred to isotope-free solutions until some labeled chromosomes were estimated to have duplicated again. Colchicine was then added to accumulate division figures, some of which contained the labeled chromosomes. These cells regularly had chromosomes with one chromatid labeled and the other free of label (Fig. 2). However, a complication was noted in the very first cells examined. A chromatid frequently would be labeled for only a part of its length, and its sister would be labeled from the point where the labeling stopped to the end or to another point of exchange. With rare exceptions, which will be discussed later, each chromosome had the equivalent of a completely labeled chromatid and a completely unlabeled chromatid when account was taken of the exchanges.

Since chromosome duplication in these cells requires several hours, the question of the sequence of synthesis along a chromosome should be subject to investigation with the autoradiographic technique. The first labeled chromosomes to arrive at division after roots have been placed in thymidine-H^3 should have spent only a part of the replication cycle in contact with the isotope. Would they be labeled throughout their entire length with less radioactivity than those arriving at divisions a few hours later? Or might they be labeled only in the small regions that duplicated after thymidine-H^3 was available? Although more investigations are required, species appear to vary in this respect. In root cells of *Crepis capallaris* small regions

appear labeled, and the regions are those adjacent to the centromeres. From this evidence chromosome duplication appears to begin at the ends of the arms and proceeds toward the centromere (Taylor, 1958c). A few groups of prophase chromosomes were found with a gradient of labeling from the ends toward the centromeres in all six chromosomes. Since the two arms of a chromosome were not necessarily equally labeled, they apparently began and finished duplication at different times. In *Vicia faba* a few cells were seen in which whole chromosomes, in a complement of labeled and partially labeled chromosomes, were without label (Woods, unpublished). On the other hand, chromosomes of *Bellevalia* are usually all labeled simultaneously along most of their length with only rare cells showing localized regions with little or no label when the remainder of the complement is labeled. Cells arriving at divisions a few hours later in both *Bellevalia* and *Crepis* have much more uniformly labeled chromosomes, which indicates that the differences in labeling are not due to intrinsic variations in DNA concentrations along the chromosomes.

Autoradiographic Studies of Sister Chromatid Exchanges

The experiments cited above show that a chromosome before duplication has two sub-units of DNA, one of which it contributes to each daughter chromatid at duplication. Are these sub-units separate structures, half-chromatids, or do they perhaps represent the two polynucleotide chains of DNA molecules with the structure and mode of replication proposed by Watson and Crick (1953)? Both DNA molecules and chromosomes have the duplex structure in common, but of course they are vastly different in size. The chromosome would more likely be an array of such molecules organized in a manner that would allow them to uncoil and separate at each duplication cycle.

The regular segregation of a new and old unit into each chromatid suggests that the units of DNA are not identical, and therefore might be complementary structures like the two chains of the DNA molecule, in which the original could act as the template for the synthesis of the new unit. At the suggestion of Dr. Max Delbrück advantage was taken of the sister-chromatid exchanges to get additional evidence on this point. In addition some evidence on the natural frequency of sister-chromatid exchanges during the mitotic cycle has been obtained.

However, a quantitative study of the exchanges is not easy. A cell with a few large, morphologically recognizable chromosomes was a primary requirement. *Bellevalia romana* ($2n = 8$) appeared to be the best material available. An additional advantage, not realized at the beginning, was the relatively more synchronous duplication of all chromosomes of *Bellevalia* compared to some other species. This reduces the number of unlabeled segments present in the first labeled chromosomes. These would be confusing in the analysis of exchanges. The exchanges had to be analyzed at the second division, after one duplication in the absence of labeled precursors. It would likewise be very important to have cells without a pool of labeled precursors remaining after the first duplication in thymidine-H^3. Fortunately, the cells that we have studied do not accumulate a pool at the concentrations of thymidine used. However, there is still a chance of finding cells which had thymidine available during the last part of a duplication and which then passed through a division and began a second duplication before depletion of the labeled precursors. By leaving roots in thymidine only six hours and then transferring them to colchicine for about thirty-six hours at 25°C., this labeling during two duplications was almost

completely avoided (see page 76 for exceptions). Under these experimental conditions, only rarely are cells, with sixteen c-metaphase or thirty-two c-anaphase chromosomes labeled, separated and flattened sufficiently for determining all the exchanges in any four chromosomes of one morphological type.

Figure 3 shows diagrammatically the sixteen c-metaphase chromosomes from one cell, the chromosomes of which duplicated with thymidine-H^3 available. At the first division after labeling, the anaphase was prevented, but the chromosomes finally separated and duplicated again. The autoradiograph was prepared at the time the cell had reached a second c-metaphase, with sixteen instead of eight chromosomes.

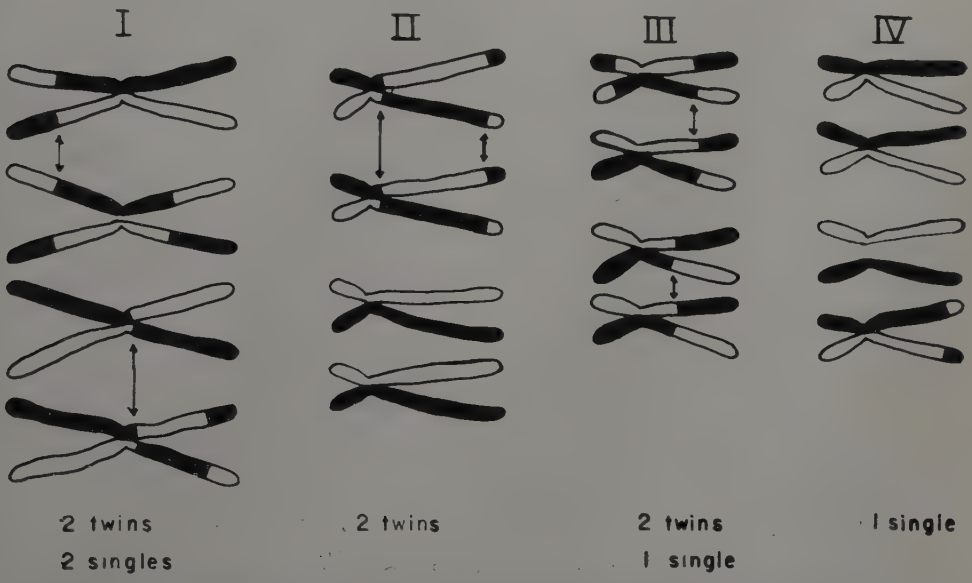


FIGURE 3. Diagrammatic sketch of the sixteen c-metaphase chromosomes at the second division after labeling with thymidine-H^3. The dark regions represent labeled parts; the regions in outline are unlabeled (after Taylor, 1958a).

TWIN AND SINGLE EXCHANGES

One of the first characteristics of the exchanges noted is their tendency to occur in pairs, that is, twin exchanges. For example, in the four large chromosomes (Fig. 3), there are exchanges in the two upper chromosomes which are at the same locus in the left arms. Likewise, in the two lower chromosomes an exchange occurs in each just to the right of the centromere. Two other exchanges have no matching exchange at homologous positions. Chromosomes with twin exchanges must have descended from the same original labeled chromosome. Therefore, the exchanges result from some rearrangement of strands that occurred before the two chromosomes separated at the first c-anaphase. If the two strands of each chromatid underwent breakage and reunion as shown in the upper diagram of Figure 4, there would result either a bridge at the following anaphase or, if the two strands of a chromatid distal to an exchange could separate and segregate with a strand of the other chromatid, unlabeled segments at the first anaphase after labeling. Since neither of these results has been observed in cells allowed to complete duplication with thymidine-H^3

available, the exchanges must involve both strands of each chromatid as shown in the lower diagram of Figure 4. The half-exchanges should also result in chromosomes with both chromatids labeled distally to the point of exchange (upper diagram, Fig. 4). Since these are very rare, the half-exchanges, if they occur at all, make no significant contribution to the frequency of sister-chromatid exchanges observed.

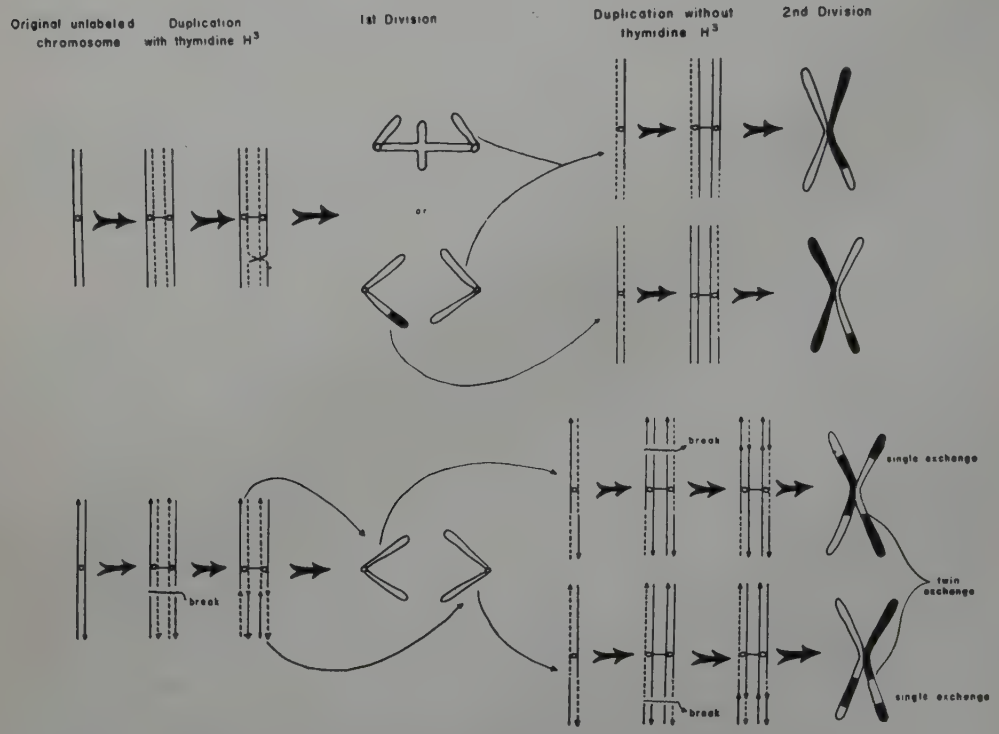


FIGURE 4. Schematic drawing to show the segregation and exchange of labeled parts of chromosomes after one duplication in thymidine-H^3. *Upper* diagram shows the results of exchanges between two of the four DNA sub-units of sister chromatids. The *lower* diagram shows the expected frequency of single and twin exchanges if a difference between the DNA sub-units exists. The difference is represented by arrows. The dashed lines and the regions of chromatids in outline represent labeled parts. Solid lines and regions in black represent unlabeled parts.

What would occur if the exchanges regularly involved all four strands of the two chromatids? First, we must consider the situation in which the two strands of a chromatid are different, as they would be if they represent complementary structures. The difference is represented for convenience by arrows to show opposite directional sense although any structural difference that would limit rejoining to like strands gives the same result (Fig. 4). The exchanges will produce no visible change at the first division; each anaphase chromosome will be labeled along the entire length. However, at the next division both chromosomes descended from such a one, with exchange and rejoining limited to like strands, will have exchanges at the same locus, that is, twin exchanges. In addition, any exchanges that occur after the second duplication will yield single exchanges. If the frequency of exchanges is equal

in the two interphases, we predict a ratio of one twin pair of exchanges to each two singles.

On the other hand, if the four strands of sister chromatids are all alike and capable of reunion in a random fashion, quite a different ratio is predicted. The original exchange at the first interphase (Fig. 5) can result in four types of reunions. One will yield a twin at the second division, one will be undetected, and two will yield single exchanges. These exchanges plus an equivalent number of exchanges at the second interphase should produce a ratio of one twin pair to ten singles. This is the minimum ratio for, if there were no difference between the two strands of a chromatid, breaks in a single chromatid at any time before the second duplication with reunion after rotation of 180° would yield a single exchange.

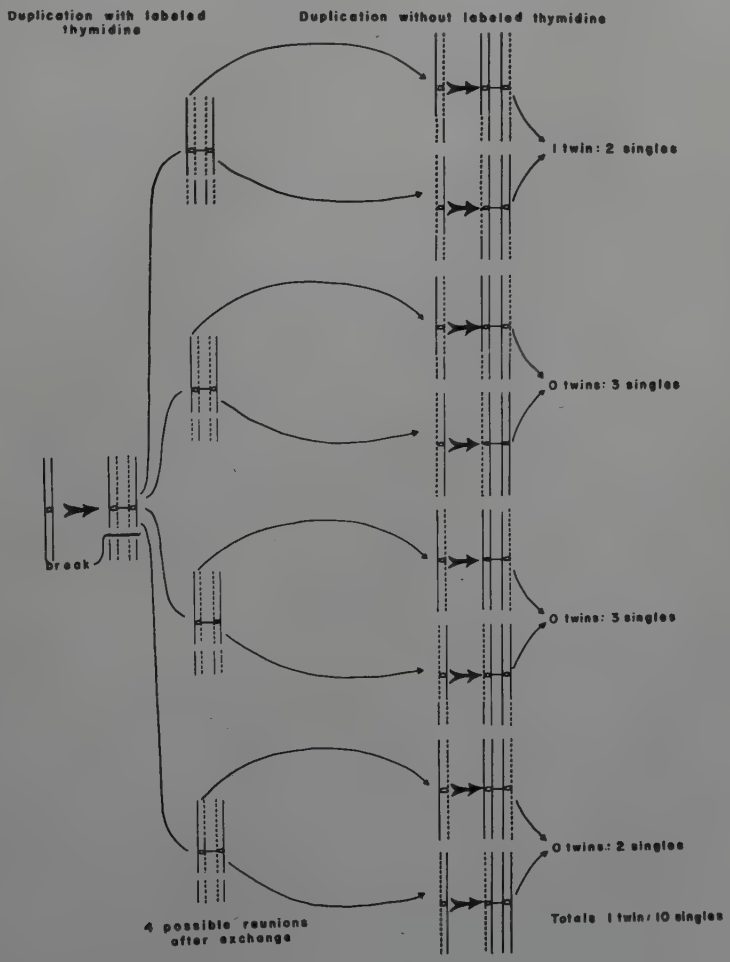


FIGURE 5. Diagram to show the expected frequency of single and twin exchanges if the two sub-units of each chromatid are alike and are capable of reunion at random. Dashed lines represent labeled parts. Exchanges following the second duplication are not shown in detail, but each chromosome is assumed to have one exchange at the second interphase after or during duplication.

First Experiment on Exchanges

In the first experiment, in which roots were placed in colchicine after six hours in thymidine-H^3, the frequency of twins to singles was greater than two to one (Table I). Data are given only for the large chromosome 1. In seventy-two chromosomes examined, four in eighteen different cells, there were seventy-two exchanges, that is, thirty-six twin pairs, plus fifteen single exchanges. This ratio does not fit either prediction. There are too many twins. However, if we assume that the hypothesis

TABLE I

Frequency of Single and Twin Exchanges in the Tetraploid Chromosome Sets of *Bellevalia romana**

Experiment†	Number of chromosomes	Twin exchanges	Single exchanges	Frequency per chromosome	
				1st interphase	2nd interphase
1	72(18 cells)	36	15	1.00	0.21
2	112(28 cells)	39	36	0.70	0.28
3	96(24 cells)	14	26	0.29	0.27

*Data for only the largest chromosome are given.
†Data for the first two experiments are from Taylor (1958a and 1958b).

of a difference between the two strands is the correct one and that all twins were produced by exchange in the first interphase and all of the singles by exchanges in the second interphase, we can calculate the frequency of exchanges (Table I). The frequency for the large chromosome is one exchange per division cycle for the first interphase, but only 0.21 exchanges during the second interphase. Some factor appears to be increasing exchanges during the first interphase or reducing them during the second interphase. Two conditions are different. Colchicine is present during the duplication in the second interphase, but not during the first. The radioactivity per chromosome is also different; there should be about twice as much in the first interphase chromosomes as in the second interphase chromosomes.

Second and Third Experiments

The factor most easily varied is the colchicine; therefore, a second experiment was carried out with colchicine supplied simultaneously with the thymidine-H^3. The ratio of twins to singles in this experiment was close to 1:1 (Table I). From this result colchicine appeared to be reducing the number of exchanges since the frequency of twins fell.

Since a lag might be occurring in the colchicine effect, a third experiment was set up in which the roots were pretreated two hours in colchicine before thymidine-H^3 was added. The ratio of twins to singles was now very near the predicted 1:2 ratio. The frequency of exchanges in the first interphase had fallen to the level occurring in the second interphase, 0.29 and 0.27, respectively.

Conclusions

From these results several conclusions can be drawn. (1) The two strands of a chromosome or a chromatid are structurally different in a way that prevents reunion

of unlike strands. (2) The frequency of exchanges is probably not significantly affected by the endogenous radiation from the tritium; otherwise the higher frequency of exchange in the first interphase should have persisted in all experiments (Taylor, 1958b). After incorporation of the thymidine-H^3 each chromosome consists of two chromatids and has two labeled strands while its descendants will have only one labeled strand at the second interphase, and therefore less endogenous radiation. (3) In the first experiment the frequency of exchanges without colchicine at first interphase is approximately the natural frequency of sister-chromatid exchanges. For the large chromosome of *Bellevalia*, this frequency is about one exchange per chromosome per division cycle. (4) The exchanges appear to occur during duplication since a two-hour pretreatment with colchicine was so much more effective in reducing the number of exchanges than simultaneous administration of colchicine and thymidine-H^3.

The Nature of the Two DNA Sub-units of Chromosomes

Since our primary concern is the organization of genetic material, let us consider the possible structural nature of the DNA sub-units of a chromosome. The autoradiographic results tell us that there are two of these units which are structurally different, as they would be if they were complementary. Are they the two polynucleotide chains of a Watson-Crick double helix? The evidence suggests that they are. However, for mechanical reasons chromosomes are likely to be arrays of molecules rather than single continuous double helices (Taylor, 1957). An experiment performed by Meselson and Stahl (1958) on the replication of DNA in *Escherichia coli* gives some very valuable clues. The treatment of cells with a detergent and centrifugation in a cesium chloride solution yields DNA particles or molecules of quite uniform size and a molecular weight of 7×10^6. This and other evidence on the disintegration of chromosomes by detergents and strong salt solutions suggests that the chromosome is an aggregate of DNA particles of rather uniform size. More important was the finding by Meselson and Stahl that the DNA of these particles behaved like that in the whole chromosome in its replication. By the use of N^{15} to produce density differences between new and original DNA, they showed with density gradient centrifugation experiments that each particle consisted of two physically continuous sub-units. After duplication each particle or molecule receives one of the sub-units which, like the sub-units in the chromosome, are conserved through many duplications. The genetic material, or chromosome if you like, is composed of a group of these particles. While Meselson and Stahl were not certain that the particles are double helices and that the DNA sub-units are single polynucleotide chains, we may at least use this concept as a working hypothesis.

The possibility has also been considered that the sub-units are double helices, that is, the DNA particles are two double helices associated into particles of 7×10^6 molecular weight. The DNA units of the chromosome, on the other hand, could not be two double helices for these would be identical rather than structurally different. The tentative conclusion then is that they are single polynucleotide chains. Could the chromosome be a bundle of these chains? The answer is that no satisfactory model has been built which will explain how the segregation of the complementary chains, new labeled and original unlabeled ones, could be regulated if the chromosomes were a bundle of DNA double helices.

Chromosome Models

Several attempts have been made to construct a workable model of a chromosome based on the structure of DNA and the information gained from autoradiography and genetic recombination (Schwartz, 1955; Bloch, 1955; Taylor, 1957, 1958d, 1958e; Freese, 1958). The first one proposed, which was based on the duplex structure of chromosomes, was the "centipede" model (Taylor, 1957, 1958e). It was proposed because it would explain how an array of DNA molecules could replicate according to the Watson-Crick scheme and segregate as shown by the experiments with thymidine-H^3. More important, the model appeared to be testable by recombination studies. If correct, certain loci on different side-chains would show non-linear linkage relations.

In addition, recombination between homologous side-arms could occur without exchange of outside markers. This property might explain certain instances of negative interference.

A limited amount of testing has shown the model to be inadequate to explain the data (Freese, 1957; Levine, 1958; Pritchard, 1957). Although the "centipede" model cannot be ruled out, a better one has been suggested (Freese, 1958; Taylor,

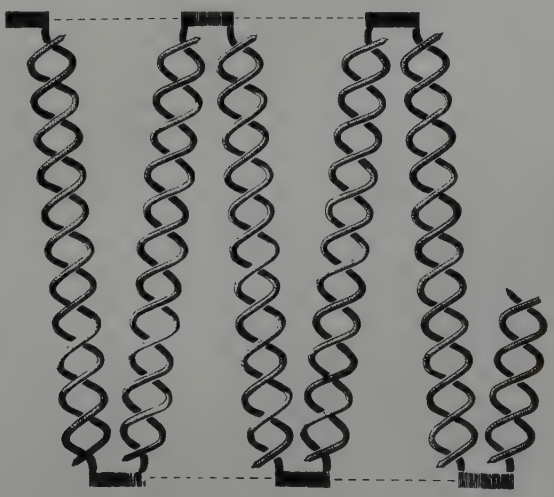


Figure 6. Diagrammatic representation of the linear model of a chromosome. The DNA particles (molecules) are linked together by protein links. The chromosome is assumed to be able to stretch out into a linear structure or to fold so that a ribbon is formed.

1958e). This duplex linear model retains many of the features of the "centipede" but places the DNA molecules in a linear array (Fig. 6). The DNA molecules are joined by blocks which we will assume are protein. The model is only a skeleton, of course, and does not account for the other proteins and the various longitudinal variations such as heterochromatin and constrictions. These features can be added when more information is available. For the present, the model can be used to explain duplication, segregation of DNA sub-units, the structural difference between the two sub-units, and genetic recombination.

First of all, the duplex linear model has the same advantages as the "centipede" in explaining the duplication and segregation of an array of DNA molecules. At one end each molecule is bonded to the linking blocks by only one polynucleotide chain and at the opposite end by the other chain. This allows the molecules to rotate freely in either direction around the single bonds and to unwind during replication. The linear model also has a difference between the two sub-units of DNA which becomes obvious when the linear structure is folded as in Figure 6. The group of polynucleotide chains attached along the upper side are complementary to those attached along the lower side. This feature was not incorporated into the "centipede" model. However, it could be added by having the DNA molecules attached by one polynucleotide chain to a single axis instead of a double one (Forro, 1957). The principal advantage of the linear model is that it appears to explain the recombination data better. In addition, it is adapted to the extreme elongation apparently necessary for the formation of lampbrush and salivary-gland chromosomes. Yet, it can fold into a ribbon, curl into a tube, and coil as explained by Taylor *et al.* (1957) and in more detail by Taylor (1958d, 1958e).

Aberrant Segregation in Tetrads

Finally, the linear model appears to explain the data on recombination in complex loci. The tendency for aberrant segregation, resulting from "copy choice" or "double replication" mechanisms, to be associated with reciprocal-type recombination is also predicted by the model. By "double replication" is meant the copying of a portion of one chromatid of a pair of homologues twice in one duplication cycle, while the homologous region in the other chromatid is not replicated at all. We must assume that this type of recombination occurs during chromosome duplication. Since duplication usually occurs before zygotene-pairing (Taylor, 1957), we assume that these are rare events which are likely to occur in localized paired regions at premeiotic interphase or at any duplication preceding a mitosis (Pontecorvo, 1958).

In the linear model, a short segment of which is shown in Figure 7, duplication may proceed from either end, but let us assume in the example shown that the chromatids are duplicating from the end with the *A* locus. After replication of the DNA molecule containing the *B* locus has passed the site of b^1, the new polynucleotide chain for some reason begins to copy off one of the chains of the homologous chromatid. If it should copy only a few nucleotides, or what is more likely, to the end of the molecule, that is, to the next protein link, as shown in Figure 7B and then wind back on the original strand which it had been copying, the result would be a "hybrid" molecule in chromatid 2. This will yield at the next replication an $A+b^2C$ and an $A++C$ chromatid; therefore, after the first postmeiotic division, the ratio of spore or cell types would be one $++$: three $+2$: four $1+$. This type of double replication should be carefully looked for in tetrad analysis. Perhaps it is a very unlikely event.

If there is to be a 3:1 segregation in the tetrad, as has been frequently reported in recent years, following Lindegren's reports, (1953; 1956) on yeast, at least one of the original strands of one chromatid must break. If the break occurs as shown in Figures 7A and 7C with reunion of an original strand with the new left strand of chromatid 1, the left strand of chromatid 2 should now replicate off the right as it unwinds from the right strand of chromatid 3. The left strand of chromatid 3 should then continue its replication off the right strand. When both the left strand of chromatid 2 and the right strand of chromatid 4 arrive at the link distal to the *B*

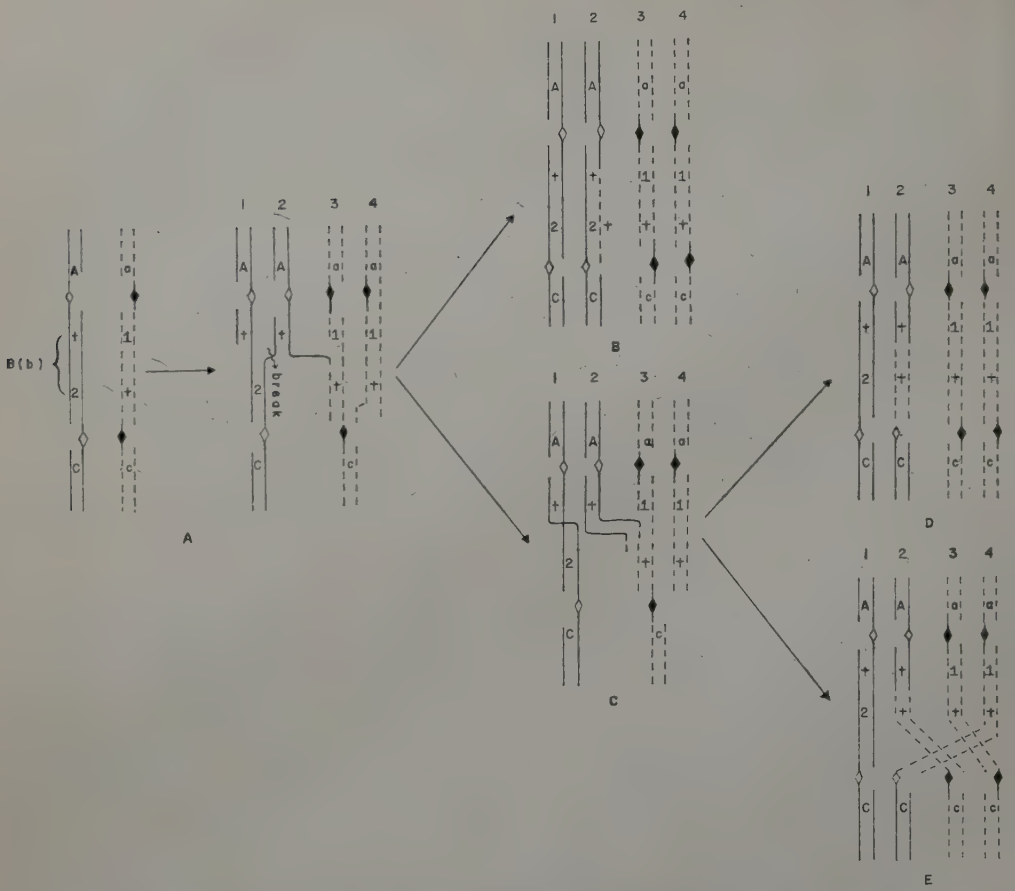


FIGURE 7. Diagram to show non-reciprocal (double replication) recombination and the predicted associated reciprocal exchanges between two duplicating chromatids. Only a very short segment of each is shown. This is the same model as that shown in Figure 6, but it is stretched out and the coiling of DNA molecules is eliminated for clarity.

locus, there may be a competition for attachment to the new connecting link. If chromatid 2 becomes attached, then chromatid 4 would have to make its attachment to the link associated with chromatid 1. The result would be a reciprocal exchange (crossover) associated with the non-reciprocal event (Fig. 7E), that is, a double replication or "conversion" as reported by Mitchell (1955a and 1955b) and Case and Giles (1958). However, if the right strand of chromatid 4 becomes attached at the new link associated with chromatid 3, the result will be a segment copied from the homologue and inserted into chromatid 2 (Fig. 7D). The first instance above might be recorded, in terms of conventional crossing-over, as a single crossover; the second instance as a double crossover, although actually the latter may be called a non-crossover. The ratio of the two events should be 1:1. The two chromatids would be $A++c$ and $A++C$. If a similar type of replication event proceeding from the other end produced a $++$ at the B locus, the chromatids would be $A++c$ and $a++c$. The triple crossover class $a++C$ would be produced very rarely when a crossover between the A and the B locus occurred in conjunction with double

replication of the type shown in Figure 7D. This class is actually, in terms of the model, a single crossover with double replication instead of a triple crossover as it would appear in a classical model of crossing-over (see the data of Freese, 1957; and Levine and Curtiss, 1958). In Table II are listed the approximate expected frequencies of chromatids with $++$ at the *B* locus, if the map distance between *A* and *B* and between *B* and *C* is in each instance two crossover units. Reciprocal classes with b^1b^2 at the *B* locus are not shown since their frequencies should be similar. Class II and III events would be increased if "double replication" of the

TABLE II

PREDICTED RECOMBINATION OF OUTSIDE MARKERS COINCIDENT WITH DOUBLE REPLICATION AMONG PSEUDO-ALLELES AT THE *B* LOCUS*

Chromatid formulae after recombination		Percentages expected	Classification of crossovers: Duplex linear model	Classification of crossovers: Conventional model
Class I	$A++c$	50.0	Single	Single
Class II	$A++C$	24.5	Non-crossover	Double
Class III	$a++c$	24.5	Non-crossover	Double
Class IV	$a++C$	1.0	Single	Triple

*Shown in Figure 7.

type shown in Figure 7B occurred, for these probably would not be associated with reciprocal exchanges. These two classes will be equal only if replication has an even chance of occurring from either end of the molecule containing the locus. The "centipede" model cannot have this property unless Forro's modification with only one polynucleotide chain attached to the axis is used. A ratio of one will not be affected very much by differences in map distances between the complex locus and the two outside markers because the associated reciprocal exchanges occur at the first link and should be much more frequent events than other non-associated reciprocal exchanges in the short region involved. The realization of this prediction should be one good test for the model; a confusing factor would be introduced, however, if replication proceeded in one direction more often than in the other. The rare Class IV events are subtracted from Class II and III, because they represent non-associated exchanges that occur in the chromatids that would have yielded Class II and Class III chromatids. The frequency of Class IV events should be equal to one-fourth the map distance between the outside markers if interference is not a factor. Some negative interference might be predicted, however, since the events are assumed to occur in regions with localized pairing; other recombination events might also have more chance of occurring in the same paired region than in other parts of the chromosome. Furthermore, if a reciprocal-type exchange occurred, the two chromatids would be held together and would be already paired at the beginning of zygotene. If one outside marker should occur on the same molecule, that is, between the *B* locus and a protein link, the frequency of single exchanges should then rise to near 75 per cent and one non-crossover class should be infrequent. In tetrad analysis the two loci should show "conversion" or double replication simultaneously.

When the data of Freese (1957) for *Neurospora* and those of Levine and Curtiss (1958) for a temperate phage are compared with those predicted by the model

(Table II), the fit is remarkably good. This agreement may be taken as evidence not only for the model, but for a similar organization of the genetic material in *Neurospora* and phage.

The above discussion leads to another question. What contribution would the reciprocal exchanges associated with "double replication" make to the total reciprocal-type recombinations? The answer undoubtedly varies with the organism. For the higher organism, the proportion would probably be small; reciprocal exchange of the conventional type may very well occur at a different time and by a different mechanism. In phage the only type of recombination may be the non-reciprocal "double replications" and the associated reciprocal exchanges.

Case and Giles (1958) report that typical reciprocal exchanges as well as non-reciprocal events occur between pseudo-alleles at the *pan* locus in *Neurospora*. If these pseudo-alleles are on one molecule, the possibility of reciprocal "copy-choice" events is presented. These require breaks of one original strand in each chromatid and would not represent double replication of one chromatid. They would add to the single crossover class, but should not affect the ratio of Class II and Class III chromatids. If one accepts the model, all reciprocal crossovers, besides such reciprocal copy-choice events, would be expected to occur at the protein links where there are single bonds. Likewise the sister-chromatid exchanges should occur at these loci. This would explain why conventional crossing-over always involves whole chromatids. It would also explain our failure to detect exchanges of only two of the four DNA sub-units in sister chromatids (Fig. 4).

Reservations and Uncertainties

After using considerable space in speculations, intriguing though they may be for those who like puzzles, we will return to reality with a few comments on classical crossing-over. In the absence of data on exchange of tritium-labeled segments during meiosis, there is little if anything new to be added. However, it should be stated that speculations on various schemes of recombination should not ignore the accumulated evidence from classical cytogenetics that reciprocal exchanges between homologous chromatids occur after zygotene-pairing in meiosis. Although this may be more a statement of faith than of fact, the conventional type of crossover in higher organisms need not be associated with the replication process and may occur at a different time and by a mechanism different from that which is associated with aberrant segregation in tetrads.

We will end this paper with one other reservation. After presenting the evidence that the DNA units of chromatids are single polynucleotide chains, a short defense of the opposing view may be in order. Rare instances of chromosomes, at the second division after labeling, with both chromatids labeled for a part of their length have been observed (Taylor, 1958a; LaCour and Pelc, 1958). These could occur as pointed out above as a result of exchanges in which the DNA sub-units acted independently of each other, that is, exchanges between an original non-labeled sub-unit and a new labeled sub-unit of the sister chromatid. However, each of these events should yield a chromosome with no label in either chromatid at a similar locus (Fig. 4). These were not observed. Another possibility to explain their occurrence is that the cells had thymidine-H^3 available for one replication cycle and for a part of another, coupled with asynchronous initiation of synthesis in the various chromosomes. A third possibility is the occurrence of several sister-chromatid exchanges in an arm of a chromosome. Because of the limits of resolu-

tion both arms will appear nearly uniformly labeled. A fourth possibility related to the one above is that the observations are meaningless because they are based on rare instances in which the various artifacts inherent in the autoradiographic technique produced the result. A final possibility recently suggested by LaCour and Pelc (1958) is that the DNA sub-units are composed of two strands which occasionally fail to segregate together. Considerably more evidence is necessary before an interpretation of the apparent instances of aberrant segregation of DNA sub-units can be evaluated.

Nevertheless, the possibility that the DNA sub-units are multiple-stranded structures (Taylor *et al.*, 1957) should not be completely excluded for higher organisms. The lack of a satisfactory model of this nature to explain replication, the presence of non-reciprocal recombination, and the idea that mutations may result from changes in individual molecules of DNA are difficult obstacles for a multi-stranded model. On the other hand, an opinion expressed by Rhoades (1959), after discussing the mutations so far intensively studied in maize and other higher organisms, conveys an idea of the state of knowledge, or lack of it, concerning the mechanism of mutation in these groups. None of the mutations has been demonstrated by cytogenetic evidence to be an intragenic change. Surely such changes occur, he adds, but they remain to be demonstrated. Therefore, we may conclude this paper with an obvious inference, namely, that various kinds of changes other than alterations in nucleotide sequence in DNA molecules may be involved in the phenomena which are called mutations.

REFERENCES

AVERY, O. T., C. M. MACLEOD, and M. MCCARTY. 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcal types: Induction of transformation by a desoxyribonucleic acid fraction isolated from *Pneumococcus* Type III. J. Exp. M. *79*: 137–158.

BENZER, S. 1957. The elementary units of heredity. *In* Symposium on the Chemical Basis of Heredity, ed. W. D. MCELROY and B. GLASS, pp. 70–93. Baltimore: Johns Hopkins Press.

BLOCH, D. 1955. A possible mechanism for the replication of the helical structure of deoxyribonucleic acid. Proc. Nat. Acad. Sc. U.S. *41*: 1058–1064.

CASE, M. E., and N. H. GILES. 1958. Evidence from tetrad analysis for both normal and aberrant recombination between allelic mutants in *Neurospora crassa*. Proc. Nat. Acad. Sc. U.S. *44*: 378–390.

FITZGERALD, P. J., M. L. EIDINOFF, J. E. KNOLL, and E. B. SIMMEL. 1951. Tritium in radioautography. Science *114*: 494–498.

FORRO, F. 1957. Personal communication, Biophysic Dept., Yale University.

FREESE, E. 1957. Ueber die Feinstruktur des Genoms im Bereich eines Pab locus von *Neurospora crassa*. Zeitsch. indukt. Abstam. vererb. *88*: 388–406.

——— 1958. The arrangement of DNA in the chromosome. Cold Spring Harbor Sympos. Quant. Biol. *23*: (in press).

FRIEDKIN, M., D. TILSON, and D. ROBERTS. 1956. Studies of deoxyribonucleic acid biosynthesis in embryonic tissues with thymidine-C^{14}. J. Biol. Chem. *220*: 627–637.

LACOUR, L. F., and S. R. PELC. 1958. The effect of colchicine on the utilization of labelled thymidine during chromosomal reproduction. Nature *182*: 506–508.

LEVINE, M., and R. CURTISS. 1958. A genetic test for side-chains in a phage chromosome. Nature *182*: 126–127.

LINDEGREN, C. C. 1953. Gene conversion in *Saccharomyces*. J. Genet. *51*: 625–637.

——— 1956. Stability of the gene. Science *124*: 26–27.

MESELSON, M., and F. W. STAHL. 1958. The replication of DNA in *Escherichia coli*. Proc. Nat. Acad. Sc. U.S. *44*: 671–682.

MITCHELL, M. B. 1955a. Aberrant recombination of pyridoxine mutants of *Neurospora*. Proc. Nat. Acad. Sc. U.S. *41*: 215–220.

——— 1955b. Further evidence of aberrant recombination in *Neurospora*. Proc. Nat. Acad. Sc. U.S. *41*: 935–937.

PRITCHARD, R. H. 1957. Personal communication, Genetics Dept., Univ. of Glasgow.
PONTECORVO, G. 1958. Trends in Genetic Analysis. New York: Columbia Univ. Press.
REICHARD, P., and B. ESTBORN. 1951. Utilization of desoxyribosides in the synthesis of polynucleotides. J. Biol. Chem. *188*: 839–846.
RHOADES, M. M. 1959. The Cytogenetics of Maize: Jessup Lectures at Columbia University for 1958. New York: Columbia Univ. Press (in press).
RIS, H. 1957. Chromosome structure. *In* Symposium on the Chemical Basis of Heredity, ed. W. D. MCELROY and B. GLASS, pp. 23–69. Baltimore: Johns Hopkins Press.
SCHWARTZ, D. 1955. Studies on crossing over in maize and *Drosophila*. J. Cellul. Physiol. *45* (suppl. 2): 171–188.
TAYLOR, J. H. 1957. The time and mode of duplication of chromosomes. Am. Natur. *91*: 209–221.
——— 1958a. Sister chromatid exchanges in tritium-labeled chromosomes. Genetics *43*: 515–529.
——— 1958b. Further studies on the mechanism of chromosome duplication. Proc. Nat. Biophys. Conf. U.S. (in press).
——— 1958c. The mode of chromosome duplication in *Crepis capillaris*. Exp. Cell. Res. *15*: 350–357.
——— 1958d. Autoradiographic studies of the organization and mode of duplication of chromosomes. *In* Symposium on Molecular Biology, ed. R. E. ZIRKLE. Chicago: Univ. of Chicago Press.
——— 1958e. The duplication of chromosomes. Sc. Am. *198*(6): 36–42.
TAYLOR, J. H., P. S. WOODS, and W. L. HUGHES. 1957. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. Proc. Nat. Acad. Sc. U.S. *43*: 122–128.
WATSON, J. D., and F. H. C. CRICK. 1953. Genetical implications of the structure of deoxyribonucleic acid. Nature *171*: 964–967.

SYMPOSIUM

ADVANCES IN HUMAN GENETICS

1. Jan A. Böök. Genetic Aspects of Schizophrenic Psychoses
2. Maurice Lamy et Jean Frézal. Etudes sur l'étiologie de la gémellité chez l'homme
3. H. Harris. Some Recent Developments in Human Biochemical Genetics
4. James V. Neel. Aspects of the Genetic Control of the Structure of the Hemoglobin Molecule
5. J. A. Fraser Roberts. Some Further Observations on Associations between Blood Groups and Disease

GENETIC ASPECTS OF SCHIZOPHRENIC PSYCHOSES

Jan A. Böök[1]

MENTAL DISEASE is recognized as one of the major public health problems of our time. Some mental conditions are undoubtedly associated with changes of cultural patterns or else with development of new environmental settings which create stress situations. Others, apparently, depend more on the large number of biological evolutionary forces which operate in human populations. Although mental breakdown may occur in any individual provided the impact of environmental forces is strong enough, individual vulnerabilities appear to be discontinuously distributed.

Among mental diseases the schizophrenic psychoses are the main causes of prolonged and serious disability. Although they rarely kill, they are significantly destructive as they claim their victims during their most productive years. In many parts of the world more than half the beds in mental hospitals are occupied by schizophrenic patients.

It is important to realize that we have no proof that schizophrenia is a disease entity with a single cause. On the contrary there are reasons to believe that we are dealing with a mental syndrome due to a variety of etiologies. I therefore prefer to talk about schizophrenic psychoses rather than about schizophrenia alone.

EPIDEMIOLOGY

When one is discussing the epidemiology of these psychoses, it is important to inquire to what extent agreement exists between different investigators as to the delimitation of the schizophrenic syndrome. At present diagnoses are based exclusively on psychological symptoms. Consequently the field is open to a variety of subjective interpretations, more or less colored by the investigator's own ideas. However, it was interesting to find at a recent conference at the World Health Organization in Geneva, which brought together psychiatrists from all parts of the world, that a fair amount of agreement could be reached on certain prominent criteria of the disease. Pending more accurate and objective diagnostic standards, differences in the classification of borderline cases must be recognized. Caution must be exercised in the interpretation of differences in incidence between different populations unless it is specifically stated that identical diagnostic principles have been used.

Although the clinical picture may be modified by cultural background, schizophrenic psychoses have been found in all the human populations which have been thoroughly investigated, irrespective of whether they enjoy a high technical culture or not: for example, the Bantus in Africa (Moffson, 1954); the Chinese on Formosa (Lin 1953); and the people of Thailand (Prasop, 1957). Schizophrenic psychoses also occur in all social strata. There is, however, some evidence which suggests a statistical association between social disorganization and schizophrenic

[1]Professor and Director, State Institute for Human Genetics, Uppsala, Sweden.

reactions. Hare (1955) and Carstairs *et al.* (1955) have shown that more hospital patients with this disease came from slums and lower social strata. Pasamanick *et al.* (1957) also found an association between psychoses and low ecomonic status. Other investigators (Clausen and Kohn, 1957; Essen-Möller *et al.*, 1956) failed to show such uneven distributions. The precise significance of these findings (cf. also Harris *et al.*, 1956) has not yet been worked out. Further studies are necessary to make possible an analysis of the complex interactions of cause and effect and of selective factors in different communities.

Epidemiological studies were initiated by genetically orientated psychiatrists in Germany early in this century. Later a number of Scandinavian geneticists and psychiatrists made significant contributions (for example, Larsson and Sjögren, 1954). Recently interest in this field has spread to people more exclusively concerned with the social and psychiatric aspects of the disease (for example, Eaton and Weil, 1955; Mayer-Gross, 1948; Bremer, 1951; and others).

For genetic purposes it is convenient to compare the morbid risks calculated in different surveys. These figures express the total risk of becoming manifestly ill for all individuals who survive the period during which the disease may appear, roughly from 15 to 50 years of age. In a large number of surveys relatively small differences have been found, the average morbid risk being about 1 per cent. Notable exceptions are the figure of 3 per cent found by Böök (1953) in a North-Swedish community and the relative rarity of schizophrenic psychoses among the American Hutterites (Eaton and Weil, 1955). Differences in the morbid risk between different populations can at present hardly be explained as due mainly to environmental variations. On the other hand, in view of the accumulated evidence that individual genetic differences are of major importance for this disease, the known distributions can be explained adequately in terms of population genetics. Consequently, any epidemiology of schizophrenic psychoses which disregards population genetics disregards essential features of this illness.

Genetic Evidence

Evidence for a genetic etiology has been accumulated in a large number of family and twin studies. As the results are very consistent, there is no need to repeat individual details here. In short, the morbid risk figures are: for siblings, 7–15 per cent; for children, 7–16 per cent; and for parents, 5–10 per cent. The figures for siblings and children are not significantly different if one compares families with one or no affected parent. Families with two affected parents have recently been studied by Elsässer (1952) who calculated a risk of about 40 per cent for the children of such couples. It is important to note that the risk of other psychoses is not significantly increased for the relatives of schizophrenic patients.

Attempts to show, by means of genetic-statistical methods, a genetic heterogeneity in terms of the common sub-groups, simplex, hebephrenic, catatonic, and paranoid forms, have so far been unsuccessful, possibly owing to small numbers when the data are broken down. Statistical associations between symptomatic groups among siblings have been reported by Schulz (1932 and 1934), Bleuler (1940), and Slater (1947). A striking symptomatic similarity was also found among the schizophrenics of a North-Swedish investigation (Böök, 1953). The latter study was planned to ensure that the material was genetically more homogeneous than that of the samples surveyed earlier. At present no definite conclusions can be drawn on the basis of these findings since such associations might equally well be due to

regional or familial environmental influences. Also, it would be hazardous to try to make genotypical divisions on the basis of psychological criteria.

Extensive twin studies by Kallmann (1946, 1950, 1953) and Slater (1953) have shown a concordance rate of 76 to 91 per cent for monozygotic and 10 to 17 per cent for dizygotic twins. In conjunction with the analysis of possible environmental causation, the results leave no reasonable doubt that the genotype is of primary importance for the development of schizophrenic psychoses.

Taken together, the family and twin data, accumulated over a period of more than forty years, are consistent enough to provide a sound basis for a genetic theory of schizophrenia. This theory does not, today, do more than imply that discontinuously distributed individual genetic differences are of decisive importance for *most* schizophrenic syndromes. It does not necessarily suggest that all such syndromes have a genetic origin or that those which do belong to one and the same genetic entity. Certainly, it does not imply that the study of the genetics is the complete answer to this illness. However, it is important to realize that so far genetics has provided the only well-founded biological starting point for further inquiries.

Mode of Inheritance

It is a common misunderstanding that genetic research has failed because no agreement has been reached as to the Mendelian mechanism of inheritance for schizophrenia. In fact, the detailed explanation of this mechanism must await further developments in diagnostic precision. Psychological diagnoses will never be suitable for the rigid statistical analysis necessary for making a decision on gene recombinations. However, the most likely explanation is that basically the schizophrenic psychoses are caused by major gene differences which express themselves regularly in homozygotes and occasionally in heterozygotes. As the heterozygotes, on this hypothesis, are quite common this should imply that more psychotics are heterozygous than homozygous. The hypothesis of a simple recessive type of transmission does not agree with the fact that no significant differences have been found between the risk figures for parents (when properly corrected according to Essen-Möller, 1955), siblings, and children with one or no affected parent. Recently, Slater (1958) has come to the same conclusion.

The explanation of the Mendelian mechanism of inheritance has to include the assumption of what is commonly called a reduced penetrance, at least in the heterozygotes. The concept of penetrance in human genetics has often been ridiculed. It has been argued that by applying the proper penetrance a reasonable fit can be obtained with an optional Mendelian ratio. This is, of course, to a certain extent true as it is also true of any senseless use of a statistical concept which was designed to describe a phenomenon but not to explain it. Penetrance is an operational and statistical concept and its nominal value is a function of diagnostic precision. Also, the effect of practically all pathological human genes is subject to considerable modifications or suppressions due to the interaction of other genes and of environmental and cultural changes.

The concordance rates for monozygotic twins may be used to calculate a penetrance which, however, has another meaning than that based on family data. When a series of schizophrenic twins is collected, the investigator, naturally, is anxious that as little doubt as possible should be raised regarding the diagnosis of the psychoses of his *propositi*. So he selects typical, often severe cases. These cases, on the present genetic hypothesis, may be assumed to belong to special entities with

high penetrance or possess genetic modifiers giving the same effect. Probably they are a mixture of both. Since the monozygotic twin siblings carry identical genes, the concordance rate and the derived penetrance will be higher than those calculated from family data. There are also a number of environmental mechanisms which work towards increasing the concordance for monozygotic twins, particularly when mental traits are considered. Consequently, the high concordance rates for schizophrenic psychoses in monozygotic twins cannot be used as an argument against the tentative explanations given above.

Whether one pair of genes or more are involved remains an open question. The point is that the distribution of schizophrenic individuals among the relatives of the *propositi* can hardly be explained otherwise than by postulating major gene differences. The clear-cut difference between psychotic and non-psychotic siblings in the absence of specific environmental causation is in favor of this interpretation. At any rate, the present data do not justify a hypothesis of polygenic inheritance in the strict sense.

Much has been written about the etiological importance of mental trauma. Although there is no reason to deny that such a causation may exist in a limited number of cases, few data have been reported that are suitable for tests to support this contention. Recently Prout and White (1956) reported a psychological study of ninety siblings of sixty schizophrenic patients. They found that mental trauma was no more common in the patients than in their siblings, although the patients reacted differently to such traumata. The hypothesis that parental deprivation is a causative factor was not substantiated. These authors conclude that the theory that trauma is a major factor in producing schizophrenic syndromes appears very questionable.

It is very unlikely that the high morbid risk of 3 per cent in the North-Swedish area (Böök, 1953) is associated with local environmental factors since no risk increase was found for the different categories of relatives of the *propositi*. The findings are best explained as an effect of selective immigration, genetic drift, or both.

As in other genetic diseases it is thought that schizophrenic psychoses are not caused exclusively by major gene differences, but that the effect of these genes is modified by other genes and, of course, by as yet unspecified environmental factors. The important conclusion which is fully justified is that major gene differences are the basic prerequisite for the initiation of a chain of events which may result in a psychosis. Unless this specific genetic prerequisite exists, the illness will not occur, provided we are not dealing with a supposedly rare non-genetic schizophrenic syndrome. Such a working hypothesis will give a firm biological basis for further research. The application of traditional genetic statistical methods to psychopathological traits has probably already yielded all the useful information it possibly can. The next step should be to concentrate on suitable approaches to the study of the somatic and biochemical effects of the major gene differences.

Biochemistry and Genetics

Although it is premature to talk about a biochemical genetics of schizophrenic psychoses, it seems pertinent to discuss some of the highlights of biochemical studies made in recent years. The increasing number of biological investigations of mental disease have been stimulated mainly by work in genetics. Schizophrenic patients have been subjected to a battery of laboratory tests, often with more imagination

and enthusiasm than skill and criticism. Nevertheless, this approach is considerably better than armchair theorizing.

The grand total of results from routine laboratory tests on schizophrenic patients has simply established a wider range of biochemical variation as compared with that in non-schizophrenic or normal individuals. A number of authors have tried to measure the toxicity of serum, urine, and spinal fluid from schizophrenics, using as test objects rabbits (Minz and Walaszek, 1957), rats (Shapiro, 1956), tadpoles (Fischer, 1953; Edisen, 1956), yeast cells (Rieder, 1952), or tissue cultures (Fedoroff, 1956; Fedoroff and Hoffer, 1956). The results are controversial. Interesting but as yet inconclusive findings have been made in the fields of endocrine dysfunction (Bleuler *et al.*, 1948; Langfeldt, 1926; and others), aromatic metabolism (McGeer *et al.*, 1956 a and b, and 1957), phosphorus metabolism (Boszormenyi-Nagy and Gerty, 1955; Boszormenyi-Nagy *et al.*, 1956), adenine metabolism (Kishimoto, *et al.*, 1955) and adrenocortical function (Hoagland *et al.*, 1955). In so far as positive findings have been made in these and other biochemical studies, schizophrenics have been reported to differ significantly as a group from normal control groups.

I should like now to consider in some detail two fields which at the moment are being intensely studied. Leach and Heath (1956) have shown that adrenaline oxidation is accelerated by fresh serum from schizophrenic patients and that this acceleration may be due to an increase of the enzyme ceruloplasmin. Elevated ceruloplasmin has also been reported by Åkerfeldt (1957), but is not characteristic of schizophrenics alone (Scheinberg *et al.*, 1957; Abood *et al.*, 1957) and its precise significance is doubtful (Aprison and Drew, 1958; Oezek, 1957). It is, therefore, thought that the oxidation of adrenaline is qualitatively different in schizophrenics. A protein fraction, called taraxein, possibly related to ceruloplasmin, has been isolated from the serum of schizophrenics. This fraction injected into humans gave rise to psychotic symptoms (Heath *et al.*, 1957). The present working hypothesis is that some schizophrenic psychoses may be caused by a genetically determined defect in those enzyme systems which maintain homeostasis by the breakdown of substances produced during stress. It remains to be seen if this applies to adrenaline metabolism. So far the results are only suggestive.

Recently serotonine has been studied in relation to schizophrenic psychoses on the basis that LSD is an anti-metabolite to this hormone and that reserpine liberates intracellular serotonine in brain tissue (Gaddum, 1953; Wooley, 1957). Serotonine itself does not produce psychotic symptoms when injected intravenously since it is incapable of passing through the blood-brain barrier. However, certain derivatives of it, such as bufotenine, n, n-dimethyltryptamine, and n, n-diethyltryptamine, cause psychosis-like symptoms in humans. Such investigations have been made mainly by biochemists and pharmacologists. Psychiatrists have remained critical, largely because experimental LSD-psychoses differ greatly from schizophrenic states and the therapeutic value of reserpine in schizophrenic psychoses is doubtful. Nevertheless, these new approaches should in a modest way be regarded as promising for the future. A number of potent substances have been found which are capable of producing profound mental changes even when present in very minute quantities.

As can be seen from this necessarily sketchy review of the vast research fields which are related to the genetics of schizophrenic psychoses, we are still far from the core of the problems. Most authors give the impression that they anticipate schizophrenia as an entity. When no consistent characteristics are found in a group of patients, the problem is dismissed and a new line taken up. It would be worth-

while studying more closely the extreme variants and not only the patients themselves but their relatives. More serious is the fact that biochemical investigations have often been carried out on patients who were not only poorly classified but were also not under proper nutritional control. It should be fairly obvious that the emotional stress that characterizes many schizophrenic patients may cause a variety of metabolic changes which have nothing to do with the real causes of the disease. Much more caution is needed in providing a more stable nutritional and emotional state before biochemical studies are carried out. At least, comparable nutritional and emotional states should be thoroughly studied in non-schizophrenic individuals.

More collaboration is needed among biochemists, psychiatrists, and geneticists. We are still waiting for the extension of biochemical investigations to monozygotic twins with only one schizophrenic partner. Etiologically important metabolic changes may be less obscure in a predisposed individual before the actual onset of the disease.

Since the various metabolic abnormalities which may be found in the blood, urine, or spinal fluid are extremely difficult to evaluate in terms of etiological significance, we are trying another approach. It seems likely that the pathology of many, perhaps all, genetic diseases is so generalized as to go down to the cellular nuclear level. On this working hypothesis it would be worthwhile studying in cell cultures the metabolism of different types of cells from schizophrenics.

When Kraepelin (1896) wrote his textbook of psychiatry some sixty years ago, he concluded that the true nature of schizophrenia was entirely obscure. Today we may reduce this statement to obscure, only, and add that the outlook for the next sixty years is, perhaps, not so dark.

REFERENCES

Abood, L. G., F. A. Gibbs, and E. Gibbs. 1957. Comparative study of blood ceruloplasmin in schizophrenia and other disorders. Arch. Neur. Psychiat., Chic. *77*: 643–645.

Akerfeldt, S. 1957. Oxidation of N, N-Dimethyl-p-phenylenediamine by serum from patients with mental disease. Science *125*: 117–119.

Aprison, M. H., and A. L. Drew. 1958. N, N-Dimethyl-p-phenylenediamine oxidation by serum from schizophrenic children. Science *127*: 758.

Bleuler, M. 1940. Krankheitsverlauf, Persönlichkeit und Verwandschaft Schizophrener und ihre gegenseitigen Beziehungen. Leipzig: Thieme.

Bleuler, M. *et al.* 1948. Untersuchungen aus dem Grenzgebiet zwischen Psychopathologie und Endokrinologie. Arch. Psychiat., Berl. *118*: 271–528.

Boszormenyi-Nagy, I., and F. J. Gerty. 1955. Difference between the phosphorus metabolism of erythrocytes of normals and of patients suffering from schizophrenia. J. Nerv. Ment. Dis. *121*: 53–59.

Boszormenyi-Nagy, I., F. J. Gerty, and J. Kueber. 1956. Correlation between an anomaly of the intracellular metabolism of adenosine nucleotides and schizophrenia. J. Nerv. Ment. Dis. *124*: 413–416.

Bremer, J. 1951. A social psychiatric investigation of a small community in northern Norway. Acta psychiat., Kbh., Supp. 62.

Böök, J. A. 1953. A genetic and neuropsychiatric investigation of a North-Swedish population, with special regard to schizophrenia and mental deficiency. Acta genet., Basel. *4*: 1–100, 133–139, 345–414.

Carstairs, G. M.; W. L. Tonge; N. O'Conner; and L. E. D. Berber. 1955. Changing population of mental hospitals. Brit. J. Prev. Social M. *9*: 187–190.

Clausen, J. A., and M. L. Kohn. 1957. Schizophrenia and the social structure of a small city. Pub. Health Rep., Wash. *72*: 578–580.

Eaton, J. W., and R. J. Weil. 1955. Culture and mental disorders: A comparative study of the Hutterites and other populations. Glencoe, Illinois. Free Press.

EDISEN, C. B. 1956. Studies of the toxicity of schizophrenic blood serum. Dis. Nerv. Syst. *17*: 77–80.

ELSÄSSER, G. 1952. Die Nachkommen geisteskranker Eltenpaare: Der Einfluss endogener Elternpsychosen auf die Psychosen, Charaktere und Liebenschicksale ihrer Kinder. Stuttgart: Thieme.

ESSEN-MÖLLER, E. 1955. The calculation of morbid risk in parents of index cases, as applied to a family sample of schizophrenics. Acta genet., Basel *5*: 334–342.

ESSEN-MÖLLER, E.; H. LARSSON; C.-E. UDDENBERG; and G. WHITE. 1956. Individual traits and morbidity in a Swedish rural population. Acta psychiat., Kbh., Supp. 100.

FEDOROFF, S. 1956. Toxicity of schizophrenics' blood serum in tissue culture. J. Laborat. Clin. M. *48*: 55–62.

FEDOROFF, S., and A. HOFFER. 1956. Toxicity of blood serum from schizophrenic and non-schizophrenic subjects. J. Nerv. Ment. Dis. *124*: 396–398.

FISCHER, R. 1953. Stress and toxicity of schizophrenic serum. Science *118*: 409–410.

GADDUM, J. H. 1953. Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. J. Physiol. *121*: 15P.

HARE, E. H. 1955. Mental illness and social class in Bristol. Brit. J. Prev. Social. M. *9*: 191–195.

HARRIS, A.; I. LINKER; V. NORRIS; and M. SHEPHERD. 1956. Schizophrenia: A prognostic and social study. Brit. J. Prev. Social M. *10*: 107–114.

HEATH, R. G.; S. MARTENS; B. E. LEACH; M. COHEN; and C. ANGEL. 1957. Effect on behavior in humans with the administration of taraxein. Am. J. Psychiat. *114*: 14–24.

HOAGLAND, H., M. RINKEL, and R. W. HYDE. 1955. Adrenocortical function and urinary phosphate excretion; comparison in schizophrenia and in lysergic acid diethylamide-induced psychotic episodes in normal persons. Arch. Neur. Psychiat. Chic. *73*: 100–109.

KALLMANN, F. J. 1946. The genetic theory of schizophrenia: An analysis of 691 schizophrenic twin index families. Am. J. Psychiat. *103*: 309–322.

——— 1950. The genetics of psychoses. Congr. Internat. Psychiat., Paris, 1950. VI. Psychiatrie sociale. Pp. 1–40.

——— 1953. Heredity in Health and Mental Disorder. New York: Norton.

KEUP, W. 1954. Die Biochemie der Schizophrenie: Eine kritische Stellungsnahme. Mschr. Psychiat. *128*: 56–90.

KISHIMOTO, K., *et al.* 1955. Biochemical genetics of schizophrenia. Report II. Annual Rep. Environ. Med. Nagoya Univ. 1955: 65–69.

KRAEPELIN, E. 1896. Psychiatrie. Leipzig: Barth.

LANGFELDT, G. 1926. The Endocrine Glands and Autonomic Systems in Dementia Praecox: Clinical and Experimental Investigations. Bergen: Eide.

LARSSON, T., and T. SJÖGREN. 1954. A methodological, psychiatric and statistical study of a large Swedish rural population. Acta psychiat., Kbh., Supp. 89.

LEACH, B. E., and R. G. HEATH. 1956. The *in vitro* oxidation of epinephrine in plasma. Arch. Neur. Psychiat., Chic. *76*: 444–450.

LIN-TSUNG-YI. 1953. A study of the incidence of mental disorder in Chinese and other cultures. Psychiatry *16*: 313–336.

MAYER-GROSS, W. 1948. Mental health survey in a rural area. Eugen. Rev., Lond. *40*: 140–148.

MCGEER, P. L., E. G. MCGEER, and W. C. GIBSON. 1956. Aromatic excretory pattern of schizophrenics. Science *123*: 1029–1030.

MCGEER, P. L., E. G. MCGEER, and J. E. BOULDING. 1956. Relation of aromatic amino acids to excretory pattern of schizophrenics. Science *123*: 1078–1080.

MCGEER, P. L.; F. E. MCNAIR; E. G. MCGEER; and W. C. GIBSON. 1957. Aromatic metabolism in schizophrenia. I. Statistical evidence for aromaturia. J. Nerv. Ment. Dis. *125*: 166–175.

MINZ, B., and E. J. WALASZEK. 1957. Sur la présence de substances biologiquement actives dans le sérum de schizophrènes. C. rend. Acad. sc. *244*: 1974–1976.

MOFFSON, A. 1954. Schizophrenia in male Bantu admissions to Weskoppies hospital. South Afr. M. J. *28*: 662–666.

OEZEK, M. 1957. Untersuchungen über den Kupferstoffwechsel im schizophrenen Formenkreis. Arch. Psychiat., Berl. *195*: 408–423.

PASAMANICK, B.; D. W. ROBERTS; P. V. LEMKAU; and D. E. KRUEGER. 1957. Mental disease prevalence in an urban population. Pub. Health Rep., Wash. *72*: 574–576.

PRASOP, R. 1957. Studies of mental illness in Thailand. Bangkok.

PROUT, C. T., and M. A. WHITE. 1956. The schizophrenic's sibling. J. Nerv. Ment. Dis. *123*: 162–170.

RIEDER, H. P. 1952. Biologische Toxizitätsbestimmung in pathologischen Körperflüssigkeiten. I. Versuche mit Nativflüssigkeiten von Geistes- und Nervenkranken. Confinia neur., Basel, *14*: 65–87.

SCHEINBERG, I. H.; A. G. MORELL; R. S. HARRIS; and A. BERGER. 1957. Concentration of ceruloplasmin in plasma of schizophrenic patients. Science *126*: 925–926.

SCHULZ, B. 1932. Zur Erbpathologie der Schizophrenie. Zbl. ges. Neur. Psychiat. *143*: 175–293.

SCHULZ, B. 1934. Versuch einer genealogisch-statistischen Ueberprüfung eines Schizophreniematerials auf biologische Einheitlichkeit. Zbl. ges. Neur. Psychiat. *151*: 145–170.

SHAPIRO, A. K. 1956. An attempt to demonstrate a catatonigenic agent in cerebrospinal fluid of catatonic schizophrenic patients. J. Nerv. Ment. Dis. *123*: 65–71.

SLATER, E. 1947. Genetical causes of schizophrenic symptoms. Mschr. Psychiat. *113*: 50–58.

——— 1958. The monogenic theory of schizophrenia. Acta genet., Basel *8*: 50–56.

SLATER, E., and J. SHIELDS. 1953. Psychotic and neurotic illnesses in twins. London: H.M.S.O. (Med. Res. Council Special Report Series, no. 278).

WOOLEY, D. W. 1957. Serotonin in mental disorders. *In* Hormones, Brain Function and Behavior, ed. H. Hoagland, p. 127. New York: Academic Press.

ÉTUDES SUR L'ÉTIOLOGIE DE LA GÉMELLITÉ CHEZ L'HOMME

Maurice Lamy et Jean Frézal[1]

Chez tous les peuples, la naissance des jumeaux est tenue pour un évènement insolite. Dans les civilisations primitives, la cause en paraît surnaturelle, mais les conséquences qui en résultent sont diverses et même opposées. Tantôt elle est considérée comme un indice du courroux divin: la mère et les enfants, ou bien les enfants seuls, sont mis à mort ou exilés. Tantôt au contraire, c'est un présage heureux qui donne lieu à des réjouissances: c'est qu'une telle naissance suggère la fertilité, la richesse, le bonheur.

C'est à la fin du siècle dernier que la recherche des causes de la gémellité est passée du domaine de la mythologie dans le champ de l'observation scientifique. Dès cette époque, les termes du problème étaient clairement posés et les recherches ultérieures n'ont pu que préciser ou modifier dans le détail, les conclusions qui avaient été tirées notamment par Duncan, par Bertillon et par Weinberg. Il suffit de relire le livre d'Apert (1923) ou celui de Dahlberg (1926) pour s'en convaincre.

La fréquence de la gémellité varie d'un pays à l'autre. Son taux est maximum en Norvège, 14.5 par 1,000, minimum au Japon 6.5. Aux Etats-Unis, la fréquence est plus élevée dans la population noire (14.3) que dans la population blanche (11.3). Ces différences de fréquence tiennent surtout à des variations de la gémellité dizygotique. La fréquence de la gémellité monozygotique paraît au contraire assez constante d'un pays à l'autre. Par exemple, les taux calculés selon la formule de Weinberg seraient respectivement pour les gémellités MZ et DZ; au Japon, 3.8 et 2.7; aux Etats-Unis, chez les blancs, 3.9 et 7.4, chez les noirs, 4.1 et 10.1.

Ces faits suggèrent *a priori* que les gémellités monozygotiques et dizygotiques relèvent de causes différentes ou du moins que le jeu réciproque de ces causes est différent pour l'une et pour l'autre. Ces causes, elles-mêmes responsables de la gémellité et des variations de sa fréquence, peuvent tenir à des différences génétiques entre les populations étudiées, à des différences de milieu ou à l'addition des deux.

Facteurs d'environnement

Parmi ces facteurs, les mieux étudiés concernent l'âge maternel et le rang de naissance. Les études qui s'y rapportent sont essentiellement fondées sur l'analyse des statistiques générales des populations, la proportion des jumeaux monozygotique étant appréciée selon la règle de Weinberg. De nombreux travaux y ont été consacrés depuis Duncan et Bertillon (Yerushalmy et Skeerar; Stokes; Enders et Stern; McArthur; Waterhouse).

La fréquence de la gémellité dizygotique est liée, à la fois et indépendamment, à l'âge maternel et au rang de naissance et peut être davantage au second facteur qu'au premier. McArthur a bien insisté sur la nécessité de tenir compte de ces deux facteurs pour les comparaisons faites d'un pays à l'autre. La courbe de fréquence en fonction de l'âge est modale et passe par un maximum pour la classe d'âge 35–39

[1]Clinique de Génétique Médicale, Hôpital des Enfants-Malades, Paris, France.

ans. Pour chaque classe d'âge, la fréquence augmente après le premier ou peut être seulement après le deuxième rang de naissance (McArthur). Il est possible que la courbe de fréquence en fonction du rang soit également modale et diminue après la huitième naissance (Waterhouse, McArthur).

L'influence de l'âge maternel sur la gémellité monozygotique est également certaine quoique moins prononcée. Sa fréquence augmente régulièrement avec celui-là jusqu'à la fin de la période de la reproduction. L'influence du rang de naissance est en revanche moins assurée. Elle serait peu importante (McArthur).

Ces faits sont illustrés sur la figure 1 qui montre les courbes de fréquence des gémellités DZ et MZ en France, pour les périodes 1902–6, 1907–10, et 1945–51.

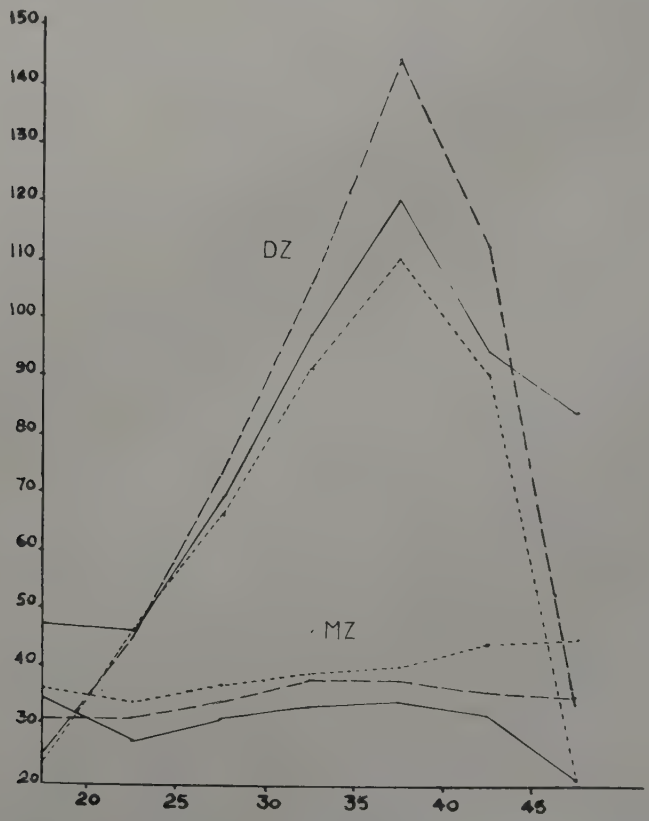


FIGURE 1. Fréquence de la gémellité en fonction de l'âge maternel en France: solid line, 1902–6; broken line, 1907–10; dotted line, 1945–52.

Il existe peu de travaux dans lesquels l'influence respective de l'âge maternel et du rang de naissance ait été recherchée dans un échantillon de jumeaux. L'avantage d'un tel matériel sur celui fourni par les statistiques de la population est de permettre un classement direct des jumeaux en fonction de la zygotie. L'inconvénient résulte des biais possibles dans la sélection des cas et tient encore à la difficulté de disposer d'un échantillon témoin comparable. L'étude que nous avons effectuée avec J. de

Grouchy et O. Chryssostomidou sur un échantillon de 1,077 jumeaux, dont 441 paires monozygotiques et 631 paires dizygotiques, nous a permis de confirmer les conclusions précédentes sur l'augmentation de fréquence des deux types de gémellité en fonction de l'âge et sur l'allure des courbes de fréquence. En revanche, nous n'avons pu déceler avec certitude une influence du rang de naissance, mais on peut faire quelques réserves sur la valeur de l'échantillon-témoin que nous avions choisi.

Outre l'âge maternel et le rang de naissance, d'autres facteurs d'environnement ont été invoqués dans l'étiologie de la gémellité. Ainsi des *facteurs socio-économiques*. Un travail de Lilienfeld et collaborateurs suggère que la fréquence de la gémellité monozygotique est plus faible dans les classes sociales les moins élevées. L'interprétation des faits reste ici extrêmement délicate. Il paraît probable que ces facteurs socio-économiques modifient la structure des familles et notamment l'âge des mères et le rang de naissance dans les différents groupes sociaux ou ethniques. Ils agissent sur la gémellité par cet intermédiaire. C'est ce qu'indique l'analyse des variations de fréquence de la gémellité. Cette étude a été faite par McArthur pour l'Australie et pour l'Italie. La fréquence de la gémellité n'était pas différente dans ces pays pour des femmes de même âge et de même parité.

Les facteurs héréditaires

L'influence des facteurs héréditaires sur la gémellité a surtout été appréciée par l'étude statistique des familles des jumeaux. En dépit de nombreux travaux, force est bien de constater que peu de progrès ont été réalisés dans nos connaissances sur ce point. Et les conclusions des études qui les concernent laissent apparaître de notables divergences.

L'opinion la plus généralement admise est que la *gémellité dizygotique* dépend de facteurs récessifs maternels comme celà semble être le cas pour les bovidés.

Les constatations de Weinberg et celles, plus récentes, de Waterhouse sont en accord avec cette hypothèse. Celles de Davenport et celles de Greulich vont à son encontre.

Il est difficile de comprendre *a priori* comment le phénomène de la polyovulation, responsable de la gémellité dizygotique, peut subir une influence paternelle, sinon selon l'hypothèse ingénieuse mais gratuite de Davenport. On la concevrait mieux en ce qui concerne les autres variétés possibles de jumeaux dizygotiques, celles qui résulteraient de la division anormale d'une ovocyte secondaire ou de la division mitotique de l'ovule qui se produit après la pénétration du spermatozoïde. En fait, l'existence de ces variétés de jumeaux reste théorique, du moins en ce qui concerne l'homme. Elle est extrêmement difficile à démontrer. Il est peu probable, leur existence admise, que ces phénomènes expliquent, en quelque façon, les constatations paradoxales faites dans les familles des jumeaux dizygotiques.

Les faits sont encore moins clairs pour la *gémellité monozygotique*. Beaucoup d'auteurs nient l'influence des facteurs génétiques. D'autres au contraire l'invoquent. Dahlberg étudie la fréquence de la survenue d'une grossesse gémellaire chez des femmes ayant donné naissance à des jumeaux. Cette fréquence est très supérieure à celle observée dans la population générale pour les jumeaux dizygotiques (4.55 ± 0.9 par 1,000). L'augmentation de fréquence de la gémellité monozygotique ne lui paraît pas en revanche assurée (1.43 ± 0.9).

L'identité ou la différence entre les facteurs génétiques éventuellement responsables des différents types de gémellité n'est pas moins discutée. Pour Dahlberg,

TABLEAU I

LES PARENTS DE JUMEAUX

Jumeaux	Total	Père jumeau Sexe du jumeau		Mère jumelle Sexe du jumeau	
		F	M	F	M
Monozygotes	490	6 (1.2%)	4 (0.8%)	3 (0.6%)	6 (1.2%)
Dizygotes	674	4 (0.6%)	12 (1.8%)	6 (1.8%)	8 (1.2%)

l'augmentation de fréquence de la gémellité chez les femmes ayant donné naissance à des jumeaux dizygotiques porte aussi bien sur la gémellité monozygotique que sur la gémellité dizygotique et inversement pour les femmes ayant eu précédemment des jumeaux monozygotiques. McArthur estime que ni la présence ni l'absence de jumeaux dans une famille n'a d'influence sur le type de la gémellité et que le degré d'association des parents avec la gémellité est indépendant de la fréquence de celle-ci dans leur descendance.

Ce rappel montre bien la complexité des faits. Pour l'illustrer encore, nous donnerons ici les constatations que nous avons faites dans un échantillon de 1,164 jumeaux classés selon la zygotie. Les fréquences de la gémellité sont très comparables chez les parents des jumeaux monozygotiques et dizygotiques, et pour chaque catégorie chez les pères et les mères, les couples unisexués et disexués (Tableau I). Il en va de même pour les différentes fréquences parmi les germains des jumeaux dizygotiques et des jumeaux monozygotiques chez qui on remarque un semblable excès de paires disexuées (Tableau II). Il est impossible de pousser plus loin cette analyse. La sélection d'un tel échantillon et notamment les raisons qui amènent des jumeaux dans une consultation spécialisée comportent, de toute évidence, de multiples biais.

Les lacunes et les échecs de telles études tiennent en premier lieu à la difficulté d'obtenir des échantillons suffisamment étendus et représentatifs. Ils tiennent aussi à la fréquence de la prédisposition à la gémellité. Pour la gémellité dizygotique supposée dépendre de facteurs maternels récessifs cette fréquence serait pour McArthur $q^2 = \frac{1}{4}$ dans la population anglaise et probablement non différente dans les populations allemandes (d'après Weinberg), ou américaines (d'après Greulich). La mise en évidence de différences significatives dans les fréquences de la gémellité chez les parents de différents degrés (relatives) des jumeaux est impossible dans des échantillons de dimensions raisonnables.

TABLEAU II

LES GERMAINS DES JUMEAUX

Jumeaux	Total	Nombre de germains	Germains jumeaux (paires) Bisexuées		Unisexuées		Total	
			n	%	*n*	%	*n*	%
Monozygotes	490	835	14	1.7	13	1.6	27	3.3
Dizygotes	674	1,360	31	2.3	26	1.9	57	4.2

On peut espérer tirer quelque enseignement des études statistiques consacrées aux facteurs d'environnement. Les déductions faites ne peuvent aboutir toutefois qu'à des conclusions négatives. Ainsi, de l'étude comparée des variations de fréquence de la gémellité en Australie et en Italie que nous avons déjà citée, McArthur concluait que la gémellité est entièrement non génétique dans son étiologie, ou que l'interaction entre les facteurs génétiques et les facteurs de milieu est identique en Australie, en Italie et probablement aussi en Angleterre. "Ceci revient à dire que la fréquence du gène est nulle ou qu'elle a une valeur constante et commune aux populations de ces pays."

Nous avons tenté d'aborder ce problème plus directement en étudiant les corrélations existant entre le taux de la consanguinité, l'âge maternel moyen et le rang de naissance pour chaque département français.

Matériel

Nous avons utilisé pour ces calculs les coefficients de consanguinité générale ($a \times 10^5$) estimés par Sutter et Tabah dans la population française pour la période 1926–45 ainsi que le pourcentage d'unions consanguines par rapport au nombre de mariages catholiques, calculé par ces mêmes auteurs pour la période 1926–30.

La fréquence de la gémellité, l'âge maternel moyen et le rang de naissance ont été calculés sur des données publiées pour chaque année par l'Institut National de la Statistique et des Etudes Economiques, pour une période de six années, 1946–51. Le nombre total des naissances en France pour cette période est de 5,892,726; celui des accouchements gémellaires est de 62,662.

Le calcul de l'âge maternel moyen et celui du rang de naissance ont été effectué pour l'ensemble des naissances, vivants et mort-nés (enfant dont la durée de gestation a dépassé six mois et qui n'ont pas respiré ou qui sont décédés avant la déclaration de naissance). Les tableaux se rapportant aux rangs de naissance sont établis suivant le nombre d'enfants vivants ou mort-nés déjà nés de la même mère. Ils ne concernant que les enfants légitimes.

Pour chaque département la proportion de jumeaux (vivants et mort-nés) dizygotiques et celle des jumeaux monozygotiques ont été évaluées selon la règle de Weinberg avec une sex-ratio égale à 0.5. L'erreur introduite est certainement négligeable. La sex-ratio calculée pour l'ensemble des jumeaux vivants et mort-nés est égale à 0.508 (1945–51) alors qu'elle est de 0.5146 pour l'ensemble des naissances en France pendant la même période.

La fréquence des accouchements multiples est égale à 10.6 (0.4) par 1,000 naissances. Les proportions respectives de jumeaux dizygotiques et monozygotiques sont 6.9 et 3.7. Les chiffres calculés sur les moyennes pondérées de chaque département sont respectivement 10.7 ; 6.97 ; et 3.70. Cependant d'assez grandes variations existent d'un département à l'autre. Les extrêmes sont, pour la gémellité totale: 15.7 (Lozère) et 8.3 (Lot et Garonne) ; pour les jumeaux dizygotiques 13.2 (Lozère) et 4.2 (Dordogne) ; pour les monozygotiques 5.2 (Ardèche) et 2.0 (Lot).

Sur la carte, la fréquence apparaît élevée en Bretagne, dans l'Est, dans les Alpes et dans la partie orientale du Massif Central. La fréquence est minimale dans le sud-ouest de la France.

Dans l'ensemble, il n'existe pas de variations brusques d'un département à l'autre mais des gradients de fréquence. Les hautes fréquences se retrouvent d'une année à

l'autre dans les mêmes régions. Enfin la carte de fréquence est très comparable à celle qui fut dressée à la fin du siècle dernier et qui est reproduite dans le livre du professeur Gedda. Une exception notable concerne l'Est du Massif Central.

Nous pensons donc que ces variations de fréquences sont réelles. Elles affectent principalement la gémellité dizygotique. La variance de cette dernière est très supérieure à celle de la gémellité monozygotique. Le rapport des variances (F) est égal à 6.5. Sa probabilité est inférieure à 1 par 1,000.

La moyenne des *âges maternels moyens* calculée pour les 90 départements français et pondérée en fonction du nombre de naissances est : $\bar{x}_1 = 28.54$ ($s = 0.15$).

Cette moyenne ne diffère pas de celle calculée pour l'ensemble de la France (1945–51) 28.58 ($s = 0.003$). Les âges extrêmes sont 27.14 (Charente Maritime) et 30.77 (Alpes-Maritimes).

Le coefficient de consanguinité moyen ($\times 10^5$) calculé dans les mêmes conditions d'après les données de Sutter et Tabah est : $\bar{x}_2 = 59.3$ ($s = 7.94$), les valeurs extrêmes de ce coefficient étant 21 (Lot et Garonne) et 236 (Corse).

Enfin *le rang de naissance* moyen est : $\bar{x}_3 = 2.5$ ($s = 0.07$), les extrêmes 1.96 (Pyrénées Orientales) et 2.97 (Doubs).

Résultats

Il existe une corrélation importante entre la fréquence de la gémellité totale et celle de la gémellité dizygotique d'une part, l'âge maternel moyen, la rang de naissance et le taux de la consanguinité d'autre part (Tableaux III et IV). Pour cette dernière nous avons calculé le corrélation en utilisant le coefficient a (1926–45) et la proportion des unions consanguines en 1926–30. Ces derniers pourcentages correspondent grossièrement aux taux de la consanguinité existant à la naissance des mères des jumeaux étudiés entre 1946 et 1951 ou du moins à ceux d'une période voisine de celle-ci. On pouvait donc s'attendre à trouver une corrélation plus étroite dans cette dernière comparaison. En fait, les valeurs calculées ne diffèrent pas les unes des autres, sans que nous puissions tirer aucune conclusion de cette constatation négative.

TABLEAU III

France 1946–52	Age maternel	Rang de naissance
Jumeaux	0.62	0.45
	(0.07)	(0.08)
D Z	0.56	0.41
	(0.07)	(0.09)
M Z	0.06	0.15
	(0.11)	(0.1)

TABLEAU IV

Jumeaux (1946–52)	Consanguinité (1926–45) (Sutter & Tabah)	Consanguinité (1926–30) (Sutter & Tabah)
Total	0.55	0.57
	(0.08)	(0.07)
Jumeaux dizygotes	0.53	0.57
	(0.08)	(0.07)
Jumeaux monozygotes	−0.03	
	(0.11)	

En revanche, il existe une différence très remarquable entre les deux variétés de gémellité. En effet, nous n'avons trouvé aucune corrélation entre la fréquence de la gémellité monozygotique et les trois facteurs étudiés. Le seul fait un peu surprenant que nous devions signaler ici, est la corrélation négative que nous avons trouvée entre les fréquences des deux variétés de gémellité, $r = -0.33$ (0.09) ; $p < 0.01$.

Il existe, d'autre part, une corrélation importante entre le taux de la consanguinité et l'âge maternel ($r = 0.59$) (Tableau V). En revanche, la corrélation entre la consanguinité et le rang de naissance ne diffère pas significativement de 0. Il en va de même de la corrélation entre l'âge maternel et le rang de naissance moyen.

TABLEAU V

	Consanguinité (α 1926-45) (Sutter & Tabah)	Rang de naissance
Age maternel	0.59 (0.07)	0.15 (0.1)
Rang de naissance	0.19 (0.09)	

Ainsi, on peut conclure de cette première analyse que la corrélation entre la fréquence de la gémellité dizygotique et le rang de naissance est réelle. Le point le plus discutable, étant donné ce que l'on sait de l'influence de l'âge maternel sur la gémellité dizygotique, concerne le rôle de la consanguinité. On peut en effet se demander si la corrélation entre la fréquence de la gémellité et le taux de la consanguinité n'est pas qu'apparente et si elle n'est pas due à la corrélation existant entre ce dernier et l'âge maternel moyen.

Pour en décider, nous avons calculé les coefficients de régression multiple de la gémellité dizygotique en fonction de l'âge maternel et de la consanguinité d'une part, et d'autre part, en fonction de ces deux caractères et du rang de naissance. La méthode utilisée pour ces calculs est celle indiquée par Brownlee (1953).

(1) *Régression de la gémellité dizygotique en fonction de l'âge et de la consanguinité* :

$y = \bar{y}\, b_1 (x_1 - \bar{x}_1) + b_2 (x_2 - \bar{x}_2)$ avec

b_1 = coefficient de régression de la gémellité dizygotique en fonction de l'âge maternel, la consanguinité étant tenue constante

b_2 = coefficient de régression en fonction de la consanguinité: on a:

$b_1 = 0.0586$

$b_2 = 0.001$

La signification de ces coefficients peut être testée par une analyse de la variance (Tableau VI).

TABLEAU VI

Origine de la variance	Somme des carrés	Degrés de liberté	Carrés moyens
Variation expliquée par X_1	136.23	1	136.23
Addition de X_2	32.06	1	32.06
Total X_1+X_2	168.29		
Reste	580.51	87	6.65
Total	748,80		

Le rapport des variances entre la valeur ajoutée par la consanguinité et le reste est égal à 4.9, sa probabilité comprise entre 0.05 et 0.01.

(2) *Régression multiple compte tenu du rang de naissance*. Nous définissons ici :
b_1 = coefficient de régression de la gémellité dizygotique en fonction de l'âge maternel, la consanguinité et le rang de naissance étant tenu constant,
b_2 et b_3 sont définis de la même façon pour la consanguinité (b_2) et le rang (b_3).
Nous avons :

$$y = \bar{y} + b_1 (x_1 - \bar{x}_1) + b_2 (x_2 - \bar{x}_2) + b_3 (x_3 - \bar{x}_3)$$

Le tableau VII montre que la prise en considération du rang de naissance modifie peu les valeurs calculées pour les coefficients de régression en fonction de l'âge maternel et du taux de consanguinité.

La somme des carrés totale est ici égale à 748.8 pour 89 degrés de liberté. La somme des carrés retirée par la régression est 251.2 pour trois degrés de liberté. Le résidu (différence entre le total et la régression) est 497.6 pour 86 degrés de liberté. La signification de chacun des coefficients de régression peut être appréciée par comparaison à son erreur standard. Il apparaît que le coefficient b_3 est hautement significatif. Le coefficient b_1 est également significatif. Le coefficient b_2 est à la limite $0.02 < p < 0.05$.

TABLEAU VII

	b	S_b	t	p
1	0.055428	0.022	2.5	$0.02 \gg 0.01$
2	0.000885	0.00042	2.1	$0.05 \gg 0.02$
3	0.157574	0.042	3.8	<0.001

Coefficients b (regression multiple de la gémellité dizygotique)

Discussion

L'analyse que nous avons faite permet d'apprécier simultanément le rôle des facteurs d'environnement et celui des facteurs héréditaires dans l'étiologie de la gémellité dizygotique. Nos résultats confirment les travaux antérieurs sur l'influence prépondérante des premiers et parmi ceux-ci, le rang de naissance paraît le plus important.

La signification du coefficient de régression calculé pour la consanguinité est plus discutable puisqu'elle se situe à un niveau de probabilité compris entre 2 et 5 par 100. Les constatations de McArthur sur la constance de la gémellité en Australie et en Italie, à âge maternel et à parité constants, doivent certes accroître nos réserves. En réalité, il eût été surprenant que nous trouvions une valeur élevée pour ce coefficient, s'il est vrai que la tendance à la gémellité dizygotique est largement répandue dans la population. C'est pourquoi nos résultats sont peut-être, en dépit de ces réserves, assez suggestifs du rôle étiologique des facteurs héréditaires récessifs dans la gémellité dizygotique, même si ce rôle paraît modeste quand on le compare à l'influence exercée par la parité et l'âge maternel.

La situation est toute différente en ce qui regarde la gémellité monozygotique. Nous n'avons trouvé aucune corrélation entre sa fréquence, le taux de la consanguinité, l'âge maternel ou le rang de naissance. Ceci est en accord avec le fait que le

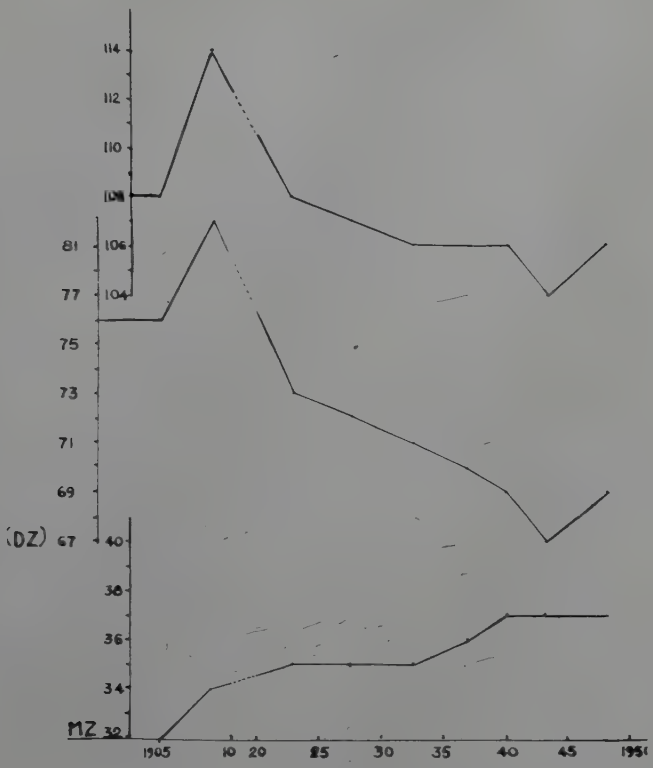


FIGURE 2. Fréquence de la gémellité en France, 1902–52.

taux de la gémellité monozygotique varie peu d'un pays à l'autre, où ces trois variables ont des valeurs absolues et relatives très différentes. De plus, ceci montre tout à la fois la sensibilité relative de la méthode d'analyse utilisée puisque l'influence de l'âge maternel semble certaine et le caractère peu marqué de cette influence.

Une telle analyse mériterait d'être complètée par l'étude des variations de fréquence de la gémellité en fonction du temps. Les courbes que nous avons tracées (Fig. 2) semblent indiquer une décroissance progressive de la gémellité dizygotique entre les deux guerres, une baisse plus brutale pendant la dernière guerre et une remontée après celle-ci. Torgensen a noté la même tendance en Norvège entre 1936 et 1948. Dans le même temps la fréquence de la gémellité monozygotique a augmenté lentement mais régulièrement.

L'analyse de ces faits est particulièrement délicate en raison des conséquences démographiques résultant des deux guerres mondiales. Nous noterons simplement d'après la statistique du mouvement de la population que la proportion des mères âgées de moins de 25 ans s'est accrue de façon continue de 1921 à 1931 « de 340 par 1,000 en 1921–5 à 361 par 1,000 en 1931. De 1931 à 1940 elle a décru jusqu'à 270 par 1,000 pour croître à nouveau par la suite. En 1945 elle est de l'ordre de 370 par 1,000 (en légère diminution sur l'année précédente). Ces variations sont étroitement liées à l'avance en âge des générations creuses de 1915 à 1919. »

Nous ne disposons pas de données précises sur le rang de naissance entre les deux guerres. Cependant, par rapport à la période 1935–7 il y a une plus forte proportion de naissances (vivants, légitimes) de rang 1 pour la période d'après guerre : de 178 en 1947 à 124 en 1951 (pour 100 en 1935–7).

McArthur a constaté une diminution légère du taux de fréquence brut de la gémellité en Italie entre les périodes 1930–2 et 1948–50.

L'augmentation de fréquence de la gémellité monozygotique en France est très comparable à celle signalée par McArthur en Australie : 3.08 (1922–4); 3.49 (1935–7) ; 3.74 (1947–9). Les causes en sont obscures. Il est possible comme le pense cet auteur, que ce phénomène tienne à une amélioration de soins prénataux

TABLEAU VIII

MORTINATALITÉ DANS LES COUPLES DE JUMEAUX DE MÊME SEXE

Années	Nombre de paires	1 mort-né	2 mort-nés
1907–10	23,835	2,527 10.6%	1,954 8.2%
1945–51	42,212	2,828 6.7%	1,143 4.7%

et à une diminution du nombre des avortements. Ceci est suggéré par la comparaison des taux de mortinatalité entre les périodes 1907–10 et 1945–51, dans la mesure où on peut inférer des taux de mortinatalité à celui des avortements. En effet, les taux de mortinatalité ont nettement diminués pour les jumeaux de même sexe (comme du reste pour les couples de sexe opposé) aussi bien pour les pourcentages des cas où les deux jumeaux étaient mort-nés que pour ceux où l'un seulement était mort-né (Tableau VIII). L'influence des facteurs d'environnement maternel dans ce phénomène est encore suggérée par les études de Karn, de Morton et de Fraccaro qui ont montré que la mortinatalité des jumeaux était en corrélation négative avec le poids de naissance. L'analyse de ce phénomène mériterait une plus ample discussion puisqu'elle varie et probablement différemment pour les couples unisexués et disexués en fonction de l'âge maternel (Lowe et Record). Notons incidemment, à ce propos, l'importance de ces analyses pour l'appréciation du rôle éventuel des "biais" signalés par Brice.

CONCLUSION

En conclusion, nous pouvons affirmer le rôle des facteurs de milieu et soupçonner celui des facteurs héréditaires dans l'étiologie de la gémellité dizygotique. La cause de la gémellité monozygotique nous échappe encore presque complètement et nous pouvons seulement la concevoir comme un accident du développement de l'œuf nanti d'une certaine probabilité et remarquablement constante dans toute l'espèce humaine.

BIBLIOGRAPHIE

APERT, E. 1923. Les jumeaux: Etude biologique, physiologique et médicale. Paris: Flammarion.

BROWNLEE, K. A. 1952. Industrial Experimentation. London: H.M.S.O.

DAHLBERG, G. 1926. Twin births and twins from a hereditary point of view. Stockholm: Bökförlags A.-B. Tidens Tryckeri.
——— 1952. Die Tendenz zu Zwillingsgeburten. Acta genet. med. gemellol., Roma *1*: 80–88.
ENDERS, T., et C. STERN. 1948. The frequencies of twins, relative to age of mothers, in American populations. Genetics *33*: 263–272.
FRACCARO, M. 1957. A contribution to the study of birth weight based on an Italian sample twin data. Ann. Human Genet., Lond. *21*: 224–236.
GEDDA, L. 1951. Studio dei gemelli. Roma: Orizzonte Medico.
GUTTMACHER, A. F. 1953. The incidence of multiple births in man and some of the other unipara. Obst. Gyn., N.Y. *2*: 22–35.
KARN, M. N. 1953. Twin data: A further study of birth weight, gestation time, maternal age, order of birth, and survival. Ann. Eugen., Lond. *17*: 233–248.
——— 1954. Data of twins born in Italy, 1936–1951. Acta genet. med. gemellol., Roma *3*: 42–49.
LAMY, M. 1949. Les Jumeaux. Paris: Correa.
LAMY, M.; J. FRÉZAL; J. DE GROUCHY; et O. CHRYSSOSTOMIDOU. 1955. L'âge maternel et le rang de naissance dans un échantillon de jumeaux. Acta genet., Basel *5*: 403–419.
LEJEUNE, J., et R. TURPIN. 1957. Influence de l'âge des parents sur la masculinité des naissances vivantes. C. rend. Acad. sc. *244*: 1833–1835.
LILIENFELD, A. M., et B. PASAMANICK. 1955. A study of variations in the frequency of twin births by race and socio-economic status. Am. J. Human Genet. *7*: 204–217.
LOWE, C. R., and R. G. RECORD. 1951. Risk of still birth in twin pregnancy related to sex and maternal age. Brit. J. Social. M. *5*: 34–40.
MCARTHUR, N. 1952. Statistics of multiple births in Italy in the years 1948–1949. Acta genet. med. gemellol., Roma *1*: 321–323.
——— 1952. A statistical study of human twinning. Ann. Eugen., Lond. *16*: 338–350.
——— 1953. The frequency of monovular and binovular twin births in Italy, 1949–1950. Acta genet. med. gemellol., Roma *2*: 11–18.
——— 1954. Changes in twin frequencies. Acta genet. med. gemellol., Roma *3*: 16–28.
MIJSBERG, W. A. 1957. Genetic-statistical data on the presence of secondary oocytary twins among non-identical twins. Acta genet., Basel *7*: 39–42.
MORTON, N. E. 1955. The inheritance of human birth weight. Ann. Human Genet., Lond. *20*: 125–134.
NOVITSKI, E., and L. SANDLER. 1957. The relationship between parental age, birth order and the secondary sex ratio in humans. Ann. Human Genet., Lond. *21*: 123–131.
PRICE, B. 1950. Primary biases in twin studies. Am. J. Human Genet. *2*: 293–352.
Statistique du mouvement de la population (France). 1945, *22*; 1946–47, *23*; 1948–49, *24*; 1950–51, *25*. Paris: Imprimerie Nationale, Presses Universitaires de France (Paris).
STOCKS, P. 1952. Recent statistics of multiple births in England and Wales. Acta genet. med. gemellol., Roma *1*: 8–13.
——— 1953. Multiple birth frequency according to parity and maternal age. Acta genet. med. gemellol., Roma *2*: 113–117.
STRANDSKOV, H. H., and J. E. ASKIN. 1953. An analysis of stillbirth frequencies among members of twin pregnancies within the total, the "white" and the "colored" populations of the United States. Acta genet. med. gemellol., Roma *2*: 164–180.
SUTTER, J., et L. TABAH. 1948. Fréquence et répartition des mariages consanguins en France. Population *3*: 607–630.
TORGENSEN, J. 1951. Heredity and environmental factors in twinning. Am. J. Phys. Anthrop. *9*: 441–454.
TURPIN, R., et M. P. SCHÜTZENBERGER. 1952. Progénèse et gémellité. Acta genet. med. gemellol., Roma *1*: 159–169.
VERSCHUER, O. v. 1957. Ueber den methodischen Beitrag der Zwillingsforschung für die Humangenetik. Acta genet. Basel *7*: 21–32.
WAARDENBURG, P. J. 1957. The twin-study method in wider perspective. Acta genet., Basel *7*: 10–20.
WALKER, N. F. 1957. Determination of the zygosity of twins. Acta genet., Basel *7*: 33–38.
WATERHOUSE, J. A. H. 1950. Twinning in twin pedigrees. Brit. J. Social M. *4*: 197–216.

SOME RECENT DEVELOPMENTS IN HUMAN BIOCHEMICAL GENETICS

H. Harris[1]

THE STUDY of inherited variation in human biochemistry can be said to have started with the work of A. E. Garrod in the early years of the present century. Garrod (1908) coined the name "inborn errors of metabolism" for a group of rare disorders which were apparently inherited and in each of which there occurred a characteristic disturbance in the normal pattern of the metabolic processes. He regarded them as examples of biochemical individuality and he thought that such individual variations in metabolism would ultimately be shown to be a common phenomenon within the species. Furthermore, he suggested that in each condition the typical biochemical and clinical findings could all eventually be traced back to a block at some point in intermediary metabolism which was probably caused by the congenital absence of a specific enzyme.

An important implication of this hypothesis, though not clearly formulated at the time, was that genetic factors may be important in determining enzyme-formation. The idea was later to become greatly elaborated and extended, and in the form of "one gene–one enzyme" became a key working hypothesis in experimental genetics. This elaboration was mainly the result of the discovery of large numbers of so-called "biochemical" mutants in micro-organisms which were shown to exhibit metabolic blocks of the kind originally envisaged by Garrod to explain the "inborn errors of metabolism" in man.

During the last few years considerable advances have been made in human biochemical genetics and it is of some interest to see how these are leading to extensions and modifications of some of the early ideas in this field. There is now little doubt that Garrod's classical concept of the "inborn errors of metabolism" as specific blocks in intermediary metabolism provides an accurate and useful approach to the study of many different types of inherited disease. Investigations in a number of conditions, such as phenylketonuria (Jervis, 1953; Wallace, Moldave, and Meister, 1957), galactosaemia (Kalckar, 1957), alkaptonuria (La Due *et al.*, 1958), and glycogen-storage disease of the liver and kidney (Cori and Cori, 1952; Harris and Olmo, 1954), have shown that not only can the characteristic and often complex peculiarities in the concentrations of various substances both intracellularly and also in the body fluids and urine of affected individuals be convincingly interpreted in terms of a single defect in intermediary metabolism, but a specific deficiency of the appropriate enzymic activity may be demonstrated directly by controlled *in vitro* studies in tissues obtained at biopsy or post mortem. Of particular interest has been the *in vitro* demonstration of a specific deficiency of amylo 1:6 glucosidase activity (Illingworth, Cori, and Cori, 1956) in one of the forms of generalized glycogenosis because it illustrates how a complex macromolecule, in this case a glycogen, may be formed with a quite unusual structure as a result of an inborn error of the classical type.

[1]Department of Biochemistry, London Hospital Medical College, London.

In many other conditions, while it has not yet been possible to study the relevant enzyme systems *in vitro*, the biochemical findings plausibly suggest that some specific enzyme deficiency exists. Furthermore, the rate at which new examples of such disorders have been reported during the last few years indicates that probably many other inherited syndromes whose biochemical basis is still not understood will ultimately be shown to exhibit this kind of metabolic block. Indeed, one not unfruitful approach to the characterization of such disorders has been the routine and rather blind search for unusual metabolites in the body fluids and urine of patients with diverse inherited syndromes in the hope of uncovering metabolic disturbances of this kind.

Recently, for example, in our own laboratory during the course of a survey of urines from patients from an institution for mentally deficient patients we encountered an individual excreting large amounts of cystathionine in her urine. The excretion appeared to be continuous and to amount to about 500 mg. per day. Cystathionine is believed to be an intermediate in the formation of cysteine from methionine. It is not present in normal urine and its occurrence in such large amounts suggested that there might be a metabolic block at the point where it is normally cleaved to give cysteine and homoserine. This hypothesis was supported by the fact that the administration of methionine to the patient led to a considerable increase in the excretion of cystathionine, and also by the finding of significantly raised concentrations of cystathionine in liver and kidney material obtained after death compared with that found in appropriate controls. Intracellular accumulation of cystathionine had evidently been occurring. The investigation of a number of relatives of the patient revealed that a clinically normal brother and also a nephew of the patient, both of whom had lived separately from the patient and under quite different conditions all their lives, also excreted significant though smaller quantities of cystathionine. Thus it seems probable that the situation represents a new genetically determined metabolic error, and no doubt now that its existence has been recognized further cases will come to light and more extensive biochemical studies will become possible.

Urine analysis is an obvious method of searching for such disorders because one may expect that a block in intermediary metabolism will often be revealed by the excretion of the metabolite immediately preceding the block, or of one of its derivatives, in unusual quantities in the urine. However, defects in intermediary metabolism are not the only cause of unusual urinary excretion patterns in inherited disease and the recognition of this has become of some practical and theoretical significance.

The excretion of large amounts of cystine and other amino acids in classical cystinuria is a case in point. This abnormality had in the past always been regarded as due to some kind of block in the intermediary metabolism either of cystine itself or of cysteine or methionine. The nature of this block had, however, long remained obscure, and when it eventually became clear that, besides cystine, the basic amino acids, lysine, arginine and ornithine, were also consistently excreted in grossly abnormal amounts by these patients, the hypothesis was even more difficult to sustain. Eventually, the demonstration that the plasma concentrations of these substances were not elevated but were, if anything, somewhat less than normal led to the suggestion by Dent that the defect was essentially renal in origin. That is to say, the grossly abnormal excretion of the four amino acids could be attributed to a failure in their reabsorption by the renal tubule cells from the glomerular filtrate

rather than from any abnormality in their intermediary metabolism. This hypothesis has now been extensively confirmed by a variety of metabolic experiments and renal clearance studies. (For a recent review, see Harris and Robson, 1957.) One must conclude that in normal individuals the process of reabsorption of amino acids by the renal tubules involves at least one step which is common to and specific for cystine, lysine, arginine, and ornithine, and that in cystinuria this is in some way defective.

Unfortunately, very little is at present known about the apparently rather complex processes which are concerned in renal tubular function. It is clear, however, that highly specific and active mechanisms are involved and it is likely that they are enzymically controlled. The defect in cystinuria can be reasonably regarded as a specific block in a renal tubular transport system and the underlying defect may well be closely analogous to that envisaged as occurring in the inborn errors of intermediary metabolism. Whether, however, it is due to a deficiency of a particular enzyme or to some other specific substance concerned in active transport remains to be seen.

The recognition of such genetically determined peculiarities in the transport of substances across the renal tubule cells raises the question whether they may not represent only a special case of a much more general phenomenon. It seems quite possible that inherited peculiarities might occur in active transport processes going on elsewhere in the body. Such processes are essential for the maintenance of many different kinds of concentration gradient across cell membranes. Abnormalities in them might well result in biochemical disturbances which could mimic very closely the kind of changes observed in blocks in intermediary metabolism of the classical type. While no certain example of such a condition has as yet been clearly delineated, the possibility must certainly be considered in connexion with obscure biochemical disorders such as Hartnup disease (Baron, *et al.*, 1956) and the new syndrome recently described by Allan *et al.* (Allan, *et al.*, 1958) in which grossly abnormal concentrations of a substance, now identified by Westall (1958) as arginosuccinic acid, were found in the cerebrospinal fluid, plasma, and urine of two mentally deficient children.

The gene-enzyme hypothesis as applied to the inborn errors of metabolism is in the first place a hypothesis about the functional character of the normal allele whose mutant form is thought to cause the abnormality in question. It implies that the normal gene at the particular locus must be present for the synthesis of a certain enzyme to occur. The mutation presumably alters the character of the gene in some way so that this function cannot be performed or is performed rather inefficiently. The hypothesis obviously only throws the problem back to a deeper level. One still has to explain what the precise role of the gene in ezyme-formation really is. Since most enzymes appear to be proteins, the problem here is likely to be part of a broader one, the problem of what role genes play in protein synthesis in general.

The nature of the functional behaviour of the mutant allele has also to be considered. In an abnormal homozygote where there is apparently a complete, or nearly complete, loss of a particular enzymic activity, such as happens in phenylketonuria or galactosaemia, is one simply dealing with a situation in which a particular enzyme protein is not formed and nothing occurs in its place, or does the synthesis occur of a protein similar to that normally encountered but differing somewhat in its structure and hence in its enzymic properties? Slight modification in a protein structure might well lead to a considerable curtailment or indeed complete loss of a particular kind of enzyme activity. It may on occasion even lead to the

appearance of new enzymic properties qualitatively different from those of its normal counterpart. It is difficult to visualize the role of gene mutations in biochemical evolution unless some process along these lines is postulated.

The recent work by Kalow and his colleagues (1957) on the genetically determined variations in the inhibition characteristics of serum cholinesterase is of particular interest in this connexion because it appears that this may be the first example in human genetics where two allelic genes control the formation of functionally active enzymes with different specificities. Attention was first directed to the occurrence of inherited differences in serum cholinesterase formation when it was observed that certain individuals are unduly susceptible to succinylcholine, a muscle relaxant often used in anaesthesia, and that the peculiarity is familial (Lehmann and Ryan, 1956). Kalow and his colleagues examined the properties of the serum cholinesterase present in such succinylcholine-sensitive people and showed that in the presence of the anticholinesterase substance, dibucaine, the percentage inhibition of its activity was only about one-fifth of that occurring with serum cholinesterase from normal people under certain standardized conditions. They believe that this peculiarity is part of an atypical pattern of substrate specificity and inhibition characteristics, and suggest that it is due to the synthesis of an enzyme protein in these individuals which differs structurally in some way from that synthesized by most other people.

Succinylcholine sensitivity occurs with a frequency of about one in a thousand to one in five thousand in the general population. However, if similar inhibition studies are carried out in a randomly chosen population, it is found that the percentage inhibition of serum cholinesterase activity with dibucaine is distributed trimodally. The rare group of succinylcholine-sensitive individuals constitutes one mode. There is also a well-defined group of individuals constituting about 3 or 4 per cent of the population with appreciably lower inhibition values than most normal people. The degree of inhibition found in this so-called "intermediate" group is, however, very much greater than that occurring in the succinylcholine-sensitive group and is in fact numerically much closer to the values found in the main group with so-called "typical" cholinesterase.

The family data reported by Kalow and Staron (1957) indicated that most individuals with the "atypical" and the "intermediate" cholinesterase phenotypes were respectively homozygous and heterozygous for a gene with a frequency of about one in sixty in the general population. Dr. Whittaker and I have also studied a series of families in which these phenotypes were segregating and so far the results fully support this hypothesis. The simplest interpretation is that the unusual allele leads to the synthesis of a protein with qualitatively different enzymic properties from that formed in the presence of the common allele. The findings in the heterozygotes correspond to what would be expected if about equal quantities of the two enzyme proteins were present (Kalow and Staron, 1957).

The search for further situations where alleles may control the formation of alternative types of enzyme is obviously important. One possible example may be the *A* and *B* genes of the ABO blood-group system. It appears probable from the work of Morgan and Kabat and their colleagues (Morgan, 1956; Kabat, 1956; Watkins and Morgan, 1955) that the water-soluble mucopolysaccharides which possess the serological specificities characteristic of this blood-group system are very similar to one another in their basic chemical structure, and that the group-specific differences depend on the presence of relatively short oligosaccharide sequences of different constitutions, formed (Watkins and Morgan, 1956) possibly

at a fairly late stage in the synthesis of the macromolecule. If so, it may be that these are determined by oligosaccharide-synthesizing enzymes with different specificities. The enzyme in group *A* individuals might, for example, be concerned in forming a particular kind of grouping in which acetylgalactosamine was a necessary unit sugar, while that in group *B* individuals was concerned with the formation of a different type of oligosaccharide sequence involving D-galactose. If this were indeed the case, the two enzymes could legitimately be regarded as products of the two allelic genes *A* and *B*. Such a concept would also fit the finding of Morgan and Watkins (1956) that *AB* heterozygotes form mucopolysaccharide molecules, all of which appear to possess both *A* and *B* specificities and are hence qualitatively different from the mucopolysaccharides found in either type of homozygote.

Any discussion of the relation of genes to enzyme-formation inevitably leads to questions about the role of genes in protein synthesis in general. In fact, one of the most interesting developments in human genetics in recent years has been the recognition of a variety of inherited differences in which there occurs some obvious peculiarity in the synthesis of a particular protein or group of proteins, and the analysis of such situations is turning out to have considerable theoretical interest. The particular proteins referred to here do not appear under normal circumstances to function as enzymes. There is, however, no reason to believe that their mode of synthesis differs in any fundamental way from the synthesis of enzyme proteins.

The series of haemoglobin variants which has been discovered during the last ten years provides the most extensively studied examples of such genetically determined differences in protein synthesis. (For recent review, see Itano, 1957.) In each variant it appears probable that a single gene difference determines the formation of a qualitatively new form of haemoglobin molecule similar in most respects to its normal counterpart but differing somewhat in certain of its physicochemical properties. Differences in the chemical structure of these alternative proteins can be expected to reflect differences in the functional activity of the alternative genes whose presence is necessary for their synthesis. So far detailed studies have been reported only for haemoglobins S and C, whose formation is controlled by genes which are probably allelic. Ingram (1957) and Ingram and Hunt (1958) have demonstrated that the essential difference between each of these haemoglobins and normal adult haemoglobin is that a single amino acid residue, out of the three hundred or so in each haemoglobin half-molecule, has been substituted by another. The arrangement and character of the remaining amino acid residues in each of the three types of molecule are probably identical.

A change as subtle as this in the haemoglobin molecule may evidently result in an alteration in its physical properties sufficient to cause major pathological and clinical disturbances. It is tempting to speculate that an analogous alteration in the fine structure of an enzyme protein could similarly produce a change in its enzymic properties and hence cause the kind of metabolic upset characteristic of the classical inborn errors of metabolism.

However, the findings in certain other inherited peculiarities in protein-formation offer a marked contrast to those obtained with the haemoglobin variants. In afibrinogenaemia (Frick and McQuarrie, 1954; Gitlin and Borges, 1953; Graham, 1956) and analbuminaemia (Bennhold, 1956), for example, the presence of a particular abnormal gene in double dose seems to result in an almost complete failure to synthesize one species of protein molecule and there is no evidence that any alternative kind of protein is found in its place. Another example is agammaglobulinaemia where affected individuals exhibit a gross though not complete failure

to form the group of proteins normally classed as gamma globulins and they are also deficient in at least two proteins normally present in the β electrophoretic fraction and which seem to be immunochemically different from gamma globulins (Gitlin, Hitzig, and Janeway, 1956).

It is easy to see how these conditions may, like the haemoglobin variants, serve as models for the enzyme deficiencies found in the classical inborn errors of metabolism and it seems clear that new methods for the analysis of enzyme proteins will be required before the relevance of such models can be evaluated.

The study of inherited variations in protein synthesis is a relatively new field and is at present expanding rapidly. The development of new techniques of protein fractionation has largely been responsible for this and the recent applications of Smithies' (1955) discovery of starch-gel electrophoresis to the investigation of plasma proteins illustrate some of the potentialities and complexities which these developments have opened up.

Smithies showed that the electrophoresis of plasma proteins in his starch-gel system led to a considerable enhancement in resolution compared with that obtained in conventional electrophoresis. Furthermore, certain differences between normal individuals, which had previously been concealed, became apparent in the new system. He demonstrated that three distinct types of protein pattern can occur, and that these are genetically determined. They depend on the properties of a group of proteins known as haptoglobins which in ordinary electrophoresis form part of the α_2 fraction and which possess the specific property of complexing with haemoglobin. The three types are now called 1.1, 2.1, and 2.2 (Smithies and Walker, 1956), and in European populations occur with a relative incidence of about 0.16, 0.48, and 0.36, respectively.

Only a single haptoglobin component can be detected in the 1.1 phenotype. A much smaller amount of a component with the same mobility as this is found in the 2.1 phenotype but none in the 2.2. However, both the 2.1 and 2.2 phenotypes show a series of components running more slowly than the 1.1 component, and of some significance is the fact that the mobilities of each of the components present in the 2.1 phenotype is different from that of any of the components in the 2.2 phenotype. Thus the pattern of haptoglobin formation is qualitatively distinct in the three types of individual.

Smithies and Walker (1955) suggested that the three haptoglobin phenotypes are determined by a pair of allelic genes, 1.1 individuals being homozygous for one of these, 2.2 individuals homozygous for the other, and 2.1 individuals being heterozygous. Galatius-Jensen (1957) has reported an extensive body of data which confirms this interpretation and in our own laboratory (Harris, Robson, and Siniscalco, 1958a) we have studied a series of nearly one hundred and fifty families, the findings in which, with very few exceptions, support this conclusion. Furthermore, the observed frequencies of the three phenotypes in a number of different populations show close agreement with the frequencies expected on this hypothesis assuming a Hardy-Weinberg equilibrium.

If this genetic hypothesis is correct, some interesting conclusions follow. A single gene substitution can evidently influence the formation of several apparently distinct though closely related proteins. Furthermore, the heterozygote may form a group of proteins qualitatively different from those present in either sort of homozygote.

However, rare exceptions to the expected pattern of segregation suggest that the genetic situation may on occasion be even more complicated. We have (Siniscalco

et al., 1958), for example, encountered one group of three related families in which five 2.2 children were derived from matings in which one of the parents was 1.1. Since in four of these the mother was the exceptional parent, illegitimacy could be excluded. It seems likely that in this family some unusual allele or modifying gene was segregating, but the precise details remain to be elucidated. Another complication is provided by the rare occurrence of individuals in whom no haptoglobins can be detected at all despite the absence of any haemolytic process which could account for this. Since this phenomenon may also be familial, a genetic causation is indicated.

Besides the examples already mentioned, a number of other inherited peculiarities in protein-formation have been recently identified. These include such biochemically diverse phenomena as the gamma globulin types of Grubb (Grubb, 1956; Grubb and Laurell, 1956), the variations in β globulin formation (Smithies, 1957, 1958; Harris, Robson, and Siniscalco, 1958b), and the abnormality in caeruloplasmin synthesis which is probably the central pathological lesion in Wilson's disease (Scheinberg and Gitlin, 1952; Scheinberg *et al.*, 1958). No doubt many more remain to be discovered and one can anticipate that in the coming years a large part of the study of inherited variation in human biochemistry will be taken up with the problems raised by such genetically determined differences in protein synthesis.

REFERENCES

Allan, J. D.; D. C. Cusworth; C. E. Dent; and V. K. Wilson. 1958. A disease, probably hereditary, characterised by severe mental deficiency and a constant gross abnormality of aminoacid metabolism. Lancet *1*: 182–187.

Baron, D. N.; C. E. Dent; H. Harris; E. W. Hart; and J. B. Jepson. 1956. Hereditary pellagra-like skin rash with temporary cerebellar ataxia, constant renal amino-aciduria, and other bizarre biochemical features. Lancet *2*: 421–428.

Bennhold, H. 1956. Kongenitale Defekt Dysproteinämien. Verh. Deut. Ges. inn. Med. *62*: 657–668.

Cori, G. T., and C. F. Cori. 1952. Glucose-6-phosphatase of the liver in glycogen storage disease. J. Biol. Chem. *199*: 661–667.

Frick, P. G., and I. McQuarrie. 1954. Congenital afibrinogenemia. Pediatrics *13*: 44–58.

Galatius-Jensen, F. 1957. Further investigations of the genetic mechanism of the haptoglobins. Acta genet., Basel *7*: 549–564.

Garrod, A. E. 1908. The Croonian lectures on inborn errors of metabolism. Lancet *2*: 1–7, 73–79, 142–148, 214–220.

Gitlin, D., and W. H. Borges. 1953. Studies on the metabolism of fibrinogen in two patients with congenital afibrinogenemia. Blood *8*: 679–686.

Gitlin, D., W. H. Hitzig, and C. A. Janeway. 1956. Multiple serum protein deficiencies in congenital and acquired agammaglobulinemia. J. Clin. Invest. *35*: 1199–1204.

Graham, J. B. 1956. Biochemical genetics of blood coagulation. Am. J. Human Genet. *8*: 63–79.

Grubb, R. 1956. Agglutination of erythrocytes coated with incomplete anti-RH by certain rheumatoid arthritic sera and some other sera; the existence of human serum groups. Acta path. microb. scand. *39*: 195–197.

Grubb, R., and A. B. Laurell. 1956. Hereditary serological human serum groups. Acta path. microb. scand. *39*: 390–398.

Harris, H., and E. B. Robson. 1957. Cystinuria. Am. J. Med. *22*: 774–783.

Harris, H., E. B. Robson, and M. Siniscalco. 1958a. Atypical segregation of haptoglobin types in man. Nature *182*: 1324–1325.

——— 1958b. β globulin variants in man. Nature *182*: 452.

Harris, R. C., and C. Olmo. 1954. Liver and kidney glucose-6-phosphatase activity in children with normal and diseased organs. J. Clin. Invest. *33*: 1204–1209.

HUNT, J. A., and V. M. INGRAM. 1958. Allelomorphism and the chemical differences of the human haemoglobins A, S and C. Nature *181*: 1062–1063.

ILLINGWORTH, B., G. T. CORI, and C. F. CORI. 1956. Amylo-1, 6-glucosidase in muscle tissue in generalized glycogen storage disease. J. Biol. Chem. *218*: 123–129.

INGRAM, V. M. 1957. Gene mutations in human haemoglobin: The chemical difference between normal and sickle cell haemoglobin. Nature *180*: 326–328.

ITANO, H. A. 1957. The human hemoglobins: Their properties and genetic control. Advance. Protein Chem. N.Y. *12*: 215–268.

JERVIS, G. A. 1953. Phenylpyruvic oligophrenia deficiency of phenylalanine-oxidizing system. Proc. Soc. Exp. Biol., N.Y. *82*: 514–515.

KABAT, E. A. 1956. Blood Group Substances: Their Chemistry and Immunochemistry. New York: Academic Press.

KALCKAR, H. M. 1957. Biochemical mutations in man and microorganisms. Science *125*: 105–108.

KALOW, W., and N. STARON. 1957. On distribution and inheritance of atypical forms of human serum cholinesterase, as indicated by dibucaine numbers. Canad. J. Biochem. Physiol. *35*: 1305–1320.

LA DUE, B. N.; V. G. ZANNONI; L. LASTER; and J. E. SEEGMILLER. 1958. The nature of the defect in tyrosine metabolism in alcaptonuria. J. Biol. Chem. *230*: 251–260.

LEHMANN, H., and E. RYAN. 1956. The familial incidence of low pseudocholinesterase level. Lancet *2*: 124.

MORGAN, W. T. J. 1956. The chemical basis of blood group specificity in man. *In* Lectures on the Scientific Basis of Medicine, vol. IV, pp. 92–111. London: Athlone Press.

MORGAN, W. T. J., and W. M. WATKINS. 1956. The product of the human blood group A and B genes in individuals belonging to group AB. Nature *177*: 521–522.

SCHEINBERG, H., and D. GITLIN. 1952. Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson's disease). Science *116*: 484–485.

SCHEINBERG, I. H.; R. S. HARRIS; A. G. MORELL; and D. DUBIN. 1958. Some aspects of the relation of ceruloplasmin to Wilson's disease. Neurology *8* (Supp. 1): 44–51.

SINISCALCO, M.; E. SILVESTRONI; I. BIANCO; H. HARRIS; and E. B. ROBSON. 1958. Hematologica (in press).

SMITHIES, O. 1955. Zone electrophoresis in starch gels: Group variations in the serum proteins of normal human adults. Biochem. J. *61*: 629–641.

——— 1957. Variations in human serum β-globulins. Nature *180*: 1482–1483.

——— 1958. Third allele at the serum β-globulin locus in humans. Nature *181*: 1203–1204.

SMITHIES, O., and N. F. WALKER. 1955. Genetic control of some serum proteins in normal humans. Nature *176*: 1265–1266.

——— 1956. Notation for serum-protein groups and the genes controlling their inheritance. Nature *178*: 694–695.

WALLACE, H. W., K. MOLDAVE, and A. MEISTER. 1957. Studies on conversion of phenylalamine to tyrosine in phenylpyruvic oligophrenia. Proc. Soc. Exp. Biol., N.Y. *94*: 632–633.

WATKINS, W. M., and W. T. J. MORGAN. 1955. Inhibition by simple sugars of enzymes which decompose the blood-group substances. Nature *175*: 676–677.

——— 1956. The A and H character of the blood group substances secreted by persons belonging to group A2. Acta genet., Basel *6*: 521–526.

WESTALL, R. G. 1958. Argininosuccinicaciduria: Identification of the metabolic defect in a newly described form of mental deficiency. Proc. IV Internat. Congr. Biochemistry (Abstracts of communications, Section 13–34, p. 168)

ASPECTS OF THE GENETIC CONTROL OF THE STRUCTURE OF THE HEMOGLOBIN MOLECULE

James V. Neel[1]

THE DISCOVERY, during the past nine years, of between fifteen and twenty inherited variations of human hemoglobin, some achieving very significant frequencies in certain populations, has placed at the disposal of students of human genetics a system providing unusual opportunities for the analysis of a variety of basic genetic problems. This presentation will be limited to a consideration of some of the very recent developments as regards the formal, biochemical, and population genetics of human hemoglobins. It should be made clear at the outset that this paper is essentially the outgrowth of a group effort, involving especially, in recent years, Dr. W. W. Zuelzer, Mr. A. Robinson, Dr. F. B. Livingstone, and Dr. F. Cohen in the United States, Dr. J. Hiernaux in the Belgian Congo, Dr. J. Linhard and Dr. G. Binson in French West Africa, and Dr. M. J. Miller in Liberia.

FORMAL GENETICS

In consequence of an informal agreement among workers in the field, the abnormal hemoglobins have been assigned letters of the alphabet in the order of their identification, unless, as for hemoglobin S (sickle-cell hemoglobin), there were overriding reasons why the usual sequence should be ignored (Neel, 1953). Table I lists the abnormal hemoglobins recognized to date. The mounting tempo of discovery is obvious. The letter A is used to designate the major component of the hemoglobin of the normal adult, and the letter F the fetal type of hemoglobin, recognized to differ from the adult type as long ago as 1866. Not indicated in the table is hemoglobin M, the letter designation supplied by Singer in 1955 for the hemoglobin abnormality in congenital methemoglobinemia, described by Hörlein and Weber in 1948. Also, the table does not include reference to the minor component of normal hemoglobin termed A_2, whose existence, previously indicated by the work of a number of investigators, was so clearly demonstrated by the study of Kunkel and Wallenius (1955), and with respect to which genetic variations are also recognized (Kunkel, Ceppellini, and Dunn, in press). Starting with hemoglobin S (Neel, 1949), the occurrence of all of these hemoglobins has been shown, with few exceptions, to be under simple genetic control. Individuals heterozygous for the abnormal gene concerned show a trait condition in which a minority of the hemoglobin is of the variant type, while in homozygous individuals all of the hemoglobin is abnormal save, in some instances, for a considerable fraction of fetal-type hemoglobin, currently regarded as a non-specific phenomenon.

Such an array of genetically controlled variations in the structure of a single protein presents an opportunity to explore the genetic basis of protein specificity. Unfortunately, progress in establishing the relationships of the various genes involved has been and will continue to be slow, since it depends on the recognition

[1]Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Mich., U.S.A.

TABLE I

HUMAN HEMOGLOBINS

Year of identification	Letter designation*	Investigators
1949	S	Pauling, Itano, Singer, and Wells
1951	C	Itano and Neel
	D	Itano
1954	E	Itano, Bergren, and Sturgeon
		Chernoff, Minnich, and Chongchareonsuk
	G	Edington and Lehmann
		Schwartz and Spaet
1955	H	Rigas, Koler, and Osgood
		Gouttas, Fessas, Tsevrenis, and Xefteri
	I	Jensen, Page, and Rucknagel
		Cabannes, Sendra, and Dalaut
1956	J	Thorup, Itano, Wheaby, and Leavell
	K	Cabannes and Buhr
		Robinson, Zuelzer, Neel, Livingstone, and Miller (Liberian 2)
	N	Robinson, Zuelzer, Neel, Livingstone, and Miller (Liberian 1)
	O	Luan Eng
1957	L	Ager and Lehmann
	P	Schneider and Haggard
1958	Q	Vella, Wells, Ager, and Lehmann

*Still undesignated are: (1) the "fast" hemoglobin of Fessas and Papaspyrou (1957); (2) the congenital sulfhemoglobinemia of Miller (1957); (3) Bart's hemoglobin of Ager and Lehmann (1958); (4) the "new" component of Gerald and Diamond (1958); (5) Hopkins I and II of Smith and Torbert (1958).

and intensive study of certain crucial families who, because of the rarity and restricted distribution of many of these hemoglobins, will be very uncommon. Because of the relatively high frequencies and overlapping distributions of hemoglobins C and S, opportunities to study the question of the allelism of the two responsible genes have been relatively common (cf. Ranney, 1954). Thus far, no instance of the independent segregation of these two genes has been recognized, and the conclusion that they are alleles seems on firm ground. The relatively few opportunities which have arisen to date to study the allelism of the other genes involved have on several occasions, listed in Table II, provided at least tentative

TABLE II

A SUMMARY OF FAMILIES PROVIDING EVIDENCE FOR THE NON-ALLELISM OF CERTAIN OF THE GENES CONCERNED WITH HEMOGLOBIN PRODUCTION

Traits	Racial background of family	Reference
A. Families providing evidence regarding two abnormal hemoglobins		
S-G	Sicilian	Schwartz, Spaet, Zuelzer, Neel, Robinson, and Kaufman, 1957
S – "Fast X"	American negro	Smith and Torbert, 1958
B. Families providing evidence regarding thalassemia and an abnormal hemoglobin		
Thal – S	Italian	Silvestroni and Bianco, 1952
Thal – S	American negro	Neel, 1951; Cohen, Robinson, Zuelzer, and Neel, in press
Thal – C	American negro	Singer, Kraus, Singer, Rubinstein, and Goldberg, 1954
Thal – C	American negro	Brown, Kloepfer, and Sprague, in press
Thal – E	Thai	Na-Nakorn and Minnich, 1957

evidence for non-allelism. It is unfortunate that such genetically important conclusions concerning allelism must for the present rest on single families, and it would undoubtedly be wise to regard these conclusions as preliminary, pending the accumulation of further evidence, since circumstances can be envisioned that would invalidate the present interpretation of these two pedigrees.

At this stage, we must provisionally recognize that at least two loci are involved in the production of human hemoglobin. If the "fast component" of Smith and Torbert ultimately proves to be inherited in genetic independence of hemoglobin G, then at least three loci are involved. It is tempting to speculate on how several different loci can collaborate in the synthesis of a single protein, especially in view of the evidence for the precision of biochemical control at one of these loci which we will be discussing later. Once the simple template hypothesis is complicated, a flood of possibilities presents itself. At the moment, there seems to be a need not for hypotheses but for more data.

It is apparent that during this phase of rapid evolution in our knowledge, an elastic and essentially descriptive system of genetic terminology is highly desirable. Such a system can be provided by the convention of using the symbol *Hb* to designate a hemoglobin locus, with the various loci being given numerical subscripts in the order of their recognition. The alleles at each locus may be designated by letter superscripts, specified by the type of hemoglobin associated with the particular allele. The terminology for the two loci thus far established is shown in Table III.

TABLE III

TERMINOLOGY FOR THE GENES CONCERNED WITH HEMOGLOBIN PRODUCTION (AFTER NEEL, 1958)

Locus	Alleles	
S-C	Normal	Hb_1^A
	Hemoglobin S	Hb_1^S
	Hemoglobin C	Hb_1^C
G	Normal	Hb_2^A
	Hemoglobin G	Hb_2^G
Thalassemia	Normal	Th^A
	Thalassemia	Th^T

Thalassemia is an inherited erythrocyte abnormality due to a gene which in the heterozygous condition produces a microcytosis with certain cytological abnormalities usually accompanied by a mild anemia, known as thalassemia minor, and in the homozygous condition, a profound anemia, known as thalassemia major, which usually terminates fatally before the age of ten. No abnormal hemoglobin has been demonstrated in this condition, but recently it has been shown that in most heterozygotes the A_2 hemoglobin component, which normally amounts to less than 3.2 per cent of the total, is elevated to as high as 8 or 9 per cent (Kunkel, Ceppellini, Müller-Eberhard, and Wolf, 1957). This discovery provides a useful handle for a diagnosis that has sometimes been elusive. Unfortunately, the technique of quantitating A_2 is sufficiently elaborate that for the moment it is beyond the routine diagnostic laboratory, and even in the research laboratory difficulties arise. Thus, an analysis of variance of the A_2 values for a series of individuals with thalassemia minor studied independently in New York City (Kunkel *et al.*, 1957), Boston (Gerald and Diamond, 1958), and Chicago (Josephson *et al.*, 1958) reveals signi-

ficant differences in the means and the variances of the three series. It seems more likely that these differences are technical rather than biological. In any given family in which thalassemia is segregating, the proportion of A_2 in affected individuals is relatively constant, suggesting the existence of a series of thalassemia alleles or, possibly, significant modifiers (Gerald and Diamond, 1958).

Some have urged that the presence of an elevated A_2 value should be considered as a prerequisite for a diagnosis of thalassemia minor (Gerald and Diamond, 1958). While it is true that the great majority of Caucasians with thalassemia minor studied thus far have shown an elevated A_2 component, our experience with thalassemia in negroes indicates that an elevated A_2 component, while common, is not as frequent as in Caucasians (Cohen, Robinson, Zuelzer, and Neel, in press). A_2 values in Mongolians with thalassemia minor have not yet been studied. It would seem premature to restrict the term thalassemia on the basis of a single biochemical finding until the worldwide situation is better understood.

Because of the wide distribution of thalassemia, opportunities to study its genetic relationships to the abnormal hemoglobins have arisen relatively frequently. Table II summarizes those studies to date which have yielded results suggesting nonallelism of a gene controlling hemoglobin production and a thalassemia gene. For technical reasons, the various studies there summarized are of uneven value, and in some instances, especially in the thalassemia–hemoglobin E family of Na-Nakorn and Minnich (1957), segregation is not the only interpretation possible. However, there seems to be no doubt that the thalassemia trait has been observed to segregate in independence of some of the genes for the abnormal hemoglobins, including the S-C locus, and it has been suggested that the locus so identified be designated as *Th*, with superscripts indicating the allele, the two thus far identified being Th^A and Th^T (Neel, 1958).

But while there is good evidence that the thalassemia trait may segregate independently of the abnormal hemoglobins, when the total data on segregation for thalassemia and hemoglobin S are reviewed, the ratios depart from expectation on the basis of complete independence. This has led to the surmise that either there is linkage or, more likely, there are at least two loci associated with the thalassemia phenotype, one allelic with the S-C locus (Neel, in press, a; Ceppellini, personal communication). Incidentally, in the family studied by Singer *et al.* (1954), which demonstrated independent segregation of thalassemia and the hemoglobin-C trait, the thalassemia was associated with findings on Tiselius electrophoresis which very likely were due to an elevated A_2 component. If there are in fact two or more different thalassemia loci, at two of which genes associated with a high A_2 fraction may occur, then obviously the A_2 criterion, while a useful guide, if rigidly adhered to may result in grouping genetically different entities while perhaps splitting off more closely related conditions.

From the fact that fetal- and adult-type hemoglobins are produced simultaneously in the fetus, and sickle- and fetal-type in the adult with sickle-cell anemia, with evidence that both types are present in a single erythrocyte, although in variable proportions (Singer and Fisher, 1952), it has been assumed that the elaboration of the two types of hemoglobin depends on different loci (Singer, Singer, and Goldberg, 1955; Huisman, Jonxis, and Van der Schaaf, 1955). In the healthy adult, not more than 1 to 2 per cent of the hemoglobin is usually of the fetal type (Singer, Chernoff, and Singer, 1951). Apparently normal Africans with fetal hemoglobin values amounting to 10 to 20 per cent of the total were recognized independently in Ghana (Edington and Lehmann, 1955) and Liberia (Neel, Hiernaux, Linhard,

Robinson, Zuelzer, and Livingstone, 1956). Jacob and Raper (1958) and Went and MacIver (1958) have shown that this characteristic is inherited as if it were due to a dominant gene. Whether this gene is an allele of the assumed fetal hemoglobin locus or involves a separate switch-over mechanism cannot be stated at present.

Recently we encountered two children in two different Liberian families who meet the diagnostic requirements for thalassemia major (Olsen, Olsen, Livingstone, Zuelzer, Robinson, and Neel, in press). Studies of the family of one of these children revealed several individuals whose hematological findings were those seen in so-called thalassemia intermedia. The genetic analysis suggests that in these particular individuals the findings are most likely due to simultaneous heterozygosity for the "high fetal" gene just mentioned and a thalassemia gene. The recognition of this new entity serves to strengthen my previous suggestion (Zuelzer, Neel, and Robinson, 1956) that most cases of so-called thalassemia intermedia represent distinct genetic entities which in no wise compromise the homozygous-heterozygous theory of the relation of thalassemia major and minor. Incidentally, the postulate of at least two different thalassemia loci provides yet another possible basis for thalassemia intermedia in individuals heterozygous for thalassemia genes at two loci.

Biochemical Genetics

A great deal of work on the precise biochemical basis for the differences by which the abnormal hemoglobins had been characterized was climaxed by the demonstration by Ingram in 1957 that hemoglobin S differed from A with respect to only a single amino acid. At one point in the sequence of nearly three hundred amino acids which comprises each of the symmetrical half-molecules of human hemoglobin, glutamic acid has been replaced by valine (Ingram, 1957). Subsequently, hemoglobin C was also shown to differ from A by a single amino acid substitution, this time of lysine, at the very same site involved in the hemoglobin-S substitution (Hunt and Ingram, 1958). In terms of current concepts of gene structure, it is impossible to avoid the surmise that the S and C genes involve alterations at very nearly if not the same purine-pyrimidine pair in the DNA chain. Hemoglobin E has also been shown to differ from normal by a single amino acid, while hemoglobins D and I are known to involve peptide changes not yet localized to single amino acids (Ingram, in press). Each of the two symmetrical, identical half-molecules of which hemoglobin is composed consists of two polypeptide chains. As long as only two hemoglobin loci have been definitely established, it is tempting to associate each with the elaboration of one of the two chains (Neel, in press, b). Ingram's data (in press) suggest that the changes in hemoglobins S, C, E, and probably one form of D involve the so-called α polypeptide chain, while the changes for hemoglobin I and probably another form of D involve the β chain. It becomes a matter of considerable relevance to the concepts of biochemical genetics to obtain data on the genetic relationships of these various genes. Thus, the hypothesis just mentioned will be in difficulty if hemoglobin G does not involve a change in the β chain.

In individuals heterozygous for the $Hb_1{}^S$ gene, the proportion of abnormal hemoglobin commonly varies between 20 and 40 per cent and this variation is to some extent under genetic control (Neel, Wells, and Itano, 1951). A comparable variation is observed in persons heterozygous for the $Hb_1{}^C$ gene. Individuals homozygous for the $Hb_1{}^S$ and $Hb_1{}^C$ genes exhibit a significant relative decrease in the ability to

produce hemoglobin (Crosby and Akeroyd, 1952; Terry, Motulsky, and Rath, 1954; Thomas, Motulsky, and Walters, 1955; James and Abbott, 1955). Itano (1953, 1955) has interpreted these facts as indicating that the "efficiency" of the abnormal gene in hemoglobin production lags behind its normal allele. From the fact that in individuals heterozygous for both these genes the two abnormal hemoglobins occur in nearly equal proportions, one could infer that the "efficiency" of these two mutant alleles is depressed to about the same extent. It is to me a very striking fact that a gene change so slight as to affect only a single amino acid can at the same time so impair what for want of a better term we call gene efficiency. Were hemoglobin an enzyme, then the $Hb_1{}^S$ gene might well appear to have both quantitative and qualitative effects.

In individuals homozygous for Th^T who have not received transfusions, the proportion of hemoglobin of type F is usually 60 per cent or higher (Zannos, 1953). The profound anemia in these patients appears to be due to a partial block in normal hemoglobin production, with persistence of the synthesis of fetal-type hemoglobin (Rich, 1952), together with an increased rate of hemolysis, taking place to a considerable extent even before the abnormally shaped erythrocytes of this disease have been released from the marrow (Sturgeon and Finch, 1957). Reasoning by analogy from what is known of the dynamics of thalassemia major, it seems quite likely that the elevation of the A_2 component observed in some cases of thalassemia minor is not entirely a true elevation but only relative, reflecting a diminished production of the principal hemoglobin component. In thalassemia major, Kunkel and collaborators (1957) find that the proportion of A_2 is not elevated with respect to the total hemoglobin present, but with reference to hemoglobin A it is as elevated as it is in thalassemia minor when the fetal component is ignored. Inasmuch as the patients they studied were undoubtedly receiving transfusions regularly, it seems quite possible that in untreated cases of thalassemia major the A_2 to A ratio tends to be even higher than in thalassemia minor. Further studies are necessary to determine if the thalassemia gene reduces the production of both A_2 and A, but of A more than A_2.

In view of the relative constancy of the clinical picture in thalassemia major, the interpretation of the biochemical genetics of the abnormal hemoglobins has been confused by the extreme variability of the biochemical "interaction" of the $Hb_1{}^S$ and Th^T genes, or of the $Hb_1{}^C$ and Th^T genes: some individuals with the two genes having predominantly an S + F or C + F hemoglobin pattern, with very little or perhaps no hemoglobin A; others showing only 25 per cent of S or C, the remainder being A. Why should the $Hb_1{}^S$ gene which, as we have just seen, on other grounds appears to have a low efficiency, in the presence of a thalassemia gene often appear to have an enhanced efficiency? Now that the possible existence of two thalassemia loci has been recognized, a way out of this dilemma would seem to present itself; one could postulate that interaction is associated with the thalassemia gene allelic to the Hb_1 locus, while non-interaction occurs when the other thalassemia locus (or loci) is involved. The available evidence does not disprove the former possibility regarding interaction. However, there is evidence that interaction may (Singer, Kraus, Singer, Rubinstein, and Goldberg, 1954; Brown, Kloepfer, and Sprague, in press) or may not (Cohen, Zuelzer, Neel, and Robinson, in press) occur where the non-allelic type of thalassemia is concerned.

It is an interesting fact that in the two families demonstrating non-allelism of thalassemia with the S-C locus, where the proportion of A_2 associated with the thalassemia has been studied or can be inferred, it has been elevated where inter-

action occurred (Singer, Kraus, Singer, Rubinstein, and Goldberg, 1954), and not elevated where there was no interaction (Cohen, Zuelzer, Neel, and Robinson, in press). In still another thalassemia–hemoglobin C family—which unfortunately yields no evidence regarding allelism—there is no factor interaction and the proportion of A_2 is not elevated (Zuelzer and Kaplan, 1954; Zuelzer, unpublished). Further data on the relationship between A_2 levels and factor interaction in this circumstance are greatly to be desired. A similar type of non-allelic factor interaction has been observed for the Hb_2 locus and thalassemia (Schwartz *et al.*, 1957). It was earlier postulated that the increase in A_2 observed in thalassemia was at least in part relative, indicating a decreased production of A. If a non-allelic type of factor interaction exists, it implies that there is a suppressant effect of the thalassemia gene on hemoglobin A production beyond that for S or C production,[2] or, for that matter, for hemoglobin-G production, an inference which certainly demands close scrutiny and intensive study because of its implications for the genetic control of protein synthesis.

Thus far we have considered the effects of the Hb_1^S and Hb_1^C genes solely in terms of an interference with total hemoglobin production somehow related to a minor change in the composition of the hemoglobin molecule, and this may indeed be an action basic to all or most of the genes associated with the production of abnormal hemoglobins. In the Hb_1^S gene, the alteration in gene structure which leads to the substitution of one amino acid for another has as a consequence at least two other important effects on the properties of the hemoglobin molecule. First, the hemoglobin molecule in both heterozygote and homozygote is much less "soluble" in the reduced form, a characteristic which separates hemoglobin S from the other abnormal hemoglobins so far recognized and which is at least in part responsible in the homozygote for intravascular sickling of the erythrocytes with all its chain of consequences (Harris, 1950). Secondly, the O_2 dissociation curve of whole blood from patients with sickle-cell anemia is shifted to the right (Becklake *et al.*, 1955; Rodman *et al.*, 1958). This alteration is in a direction to reinforce the ill effects resulting from the low solubility of hemoglobin S in the reduced state.

A possible second effect of the Hb_1^S gene is the hyposthenuria observed in individuals both homozygous and heterozygous for this gene (McCrory *et al.*, 1953; Kunz *et al.*, 1954; Zarafonetis *et al.*, 1955; Scott *et al.*, 1955; McMaster *et al.*, 1956; and Keitel and Thompson, 1956). This defect has been shown to be associated with the presence of circulating erythrocytes containing hemoglobin S, but how such erythrocytes influence renal tubular function is unknown (Keitel, Thompson, and Itano, 1956).

That there are still other effects of Hb_1^S seems quite likely. Thus, individuals heterozygous for both Hb_1^S and the gene responsible for the high fetal component described earlier often show no ill effects, although on electrophoresis their hemoglobin is indistinguishable from that of individuals with sickle-cell anemia. The possibility must be seriously considered that something more than abnormal hemoglobin is involved in the clinical state known as sickle-cell anemia.

Population Genetics

The localized distributions and relatively high frequencies of some of the genes resulting in abnormal hemoglobin production pose very puzzling problems in population genetics, problems presenting interesting analogies with those of classical

[2]Or, less likely, a positively synergistic effect on the production of the abnormal hemoglobin.

epidemiology (cf. Neel, 1957). Inasmuch as individuals homozygous for the genes associated with hemoglobins S, C, and E all carry appreciable biological handicaps, considerable interest has converged on the possibility that these genes are members of balanced polymorphic systems, especially in view of the evidence that even the *maximum* mutation rate for the $Hb_1{}^S$ gene in Africa is far below that which could maintain the observed gene frequency (Vandepitte *et al.*, 1955). Allison (1954a, 1954b) in a series of papers has developed the arguments for regarding a decreased susceptibility to *P. falciparum* malaria on the part of individuals heterozygous for the $Hb_1{}^S$ gene as the factor responsible for maintaining polymorphism, and what seems to be critical evidence in favor of the hypothesis has now been supplied by Raper (1956) and Vandepitte and Delaisse (1957). A possible physiological basis for this differential susceptibility, involving the intravascular sickling of parasitized, deoxygenated erythrocytes, has been suggested by our group (Miller, Neel, and Livingstone, 1956). No physiological basis for maintaining the frequencies of individuals with hemoglobin C or E has yet been developed.

Until very recently, the distribution of thalassemia was thought to center about the Mediterranean basin. Then, several years ago, it became apparent that there was a second focus of the disease in Thailand (Minnich, Na-Nakorn, Chongchareonsuk, and Kochaseni, 1954). More recently, at the Conference on Abnormal Hemoglobins held in Turkey in 1957, it became apparent that there are many areas of high thalassemia frequency between Thailand and the Mediterranean basin, so that there is indeed an extensive and continuous distribution of the responsible gene. It is worth re-emphasizing (cf. Neel and Valentine, 1947) that since the individual heterozygous for the thalassemia gene is undoubtedly under a significant hematological handicap, any differential disease susceptibility which serves as a means of maintaining this polymorphism must be a rather significant one.

The fact that the genes we have been discussing are apparently associated with phenotypes subjected to rather significant and variable selection (not to mention mutation) indicates that caution must be exercised in using their occurrence and frequency in two groups as easy indicators of relationship. With this admonition, I should like to consider briefly the light which studies on the abnormal hemoglobins have thrown on the history of the tribes of Liberia and the adjacent Ivory Coast, as an example of the potential use of the hemoglobins in such problems. Although Liberia is an intensely malarious region, only 8.9 per cent of a large series of natives tested by Livingstone (1958) exhibited the sickling phenomenon, rather than the 30 to 40 per cent postulated as the equilibrium value in regions with hyperendemic *falciparum* malaria (Allison, 1954). There is, moreover, a striking cline in this frequency, the tribes of northwestern Liberia exhibiting a frequency of 15 to 20 per cent of the sickling phenomenon, the tribes of southeastern Liberia frequencies of 0 to 1 per cent. These low frequencies are characteristic of the adjacent eastern Ivory Coast, but the frequencies then rise as one approaches Ghana (Zuelzer, Neel, Binson, and Robinson, unpublished). The frequency of the $Hb_1{}^C$ gene is also lower in Liberian tribes than in those of countries to both the east and the west, such as Ghana and Sierra Leone (Neel *et al.*, 1956). On the other hand, the hemoglobin now known as N was encountered in seven out of 904 individuals, representing all the tribes of Liberia. All but one of these seven individuals were from the Mande-speaking tribes of the interior of central Liberia (Loma, Kpelle, Mano, Gio); in these tribes the frequency of individuals with this abnormal hemoglobin was about 2 per cent. In spite of rather extensive work on the abnormal hemoglobins in West Africa, this hemoglobin has only been reported in two other individuals, one from

Ghana and one from Nigeria (Ager and Lehmann, 1958). The two Liberian cases of thalassemia major mentioned above were encountered in the relatively small hospital maintained by the Firestone Company at its plantation in the southeastern section of the country (Olsen, Olsen, Livingstone, Zuelzer, Robinson, and Neel, in press). On the basis of doing starch-block electrophoresis *only after paper electrophoresis suggested an elevation of the* A_2 *component*, a first approximation to the minimal frequency of thalassemia minor is 11 per cent. Despite extensive medical facilities throughout Africa, only one other case of thalassemia major has been reported south of the Sahara; this in the Belgian Congo (Vandepitte, in press). It seems quite possible, then, that the Th^T gene will be found to have a higher frequency in some of the tribes of Liberia than in those of surrounding regions. Thus, on the basis of inherited variations of the erythrocytes some of the tribes of Liberia appear to exhibit a greater affinity with those of North Africa than with their more immediate neighbors. Cultural anthropologists have long ascribed to the tribes of southeastern Liberia and the western Ivory Coast "paleonegroid" characteristics (Baumann and Westermann, 1948), these tribes having apparently been compressed into this less desirable area of dense rain forest by their more successful neighbors. The biological data now emerging on the basis of hemoglobin studies not only confirm this impression but give every promise of significantly extending our knowledge.

Concluding Remarks

This brief presentation has of necessity given only a very fragmentary view of a rapidly evolving and expanding field. Many developments of significance have been omitted. It seems certain that by the time of the next International Congress many of our current concepts and speculations will have undergone significant revision, not only because of work on man but also through work on the variant hemoglobins now being recognized in such diverse animal species as chickens, sheep, goats, cattle, buffaloes, horses, and monkeys.

REFERENCES

Ager, J. A. M., and H. Lehmann. 1957. Haemoglobin L: a new haemoglobin found in a Punjabi Hindu. Brit. M. J. *2*: 142–143.

——— 1958. Observations on some "fast" haemoglobins: K, J, N, and "Bart's." Brit. M. J. *1*: 929–931.

Allison, A. C. 1954a. Protection afforded by sickle-cell trait against subtertian malarial infection. Brit. M. J. *1*: 290–294.

——— 1954b. The distribution of the sickle-cell trait in East Africa and elsewhere, and its apparent relationship to the incidence of subtertian malaria. Tr. R. Soc. Trop. M. Hyg., Lond. *48*: 312–318.

Baumann, H. 1948. Les peuples et les civilisations de l'Afrique. Suivi de Les langues et l'éducation par D. Westermann. Paris: Payot.

Becklake, M. R.; S. B. Griffiths; M. McGregor; H. I. Goldman; and J. P. Schreve. 1955. Oxygen dissociation curves in sickle cell anemia and in subjects with the sickle cell trait. J. Clin. Invest. *34*: 751–755.

Brown, C. L., H. W. Kloepfer, and C. C. Sprague. A new sydrome: Hemoglobin CS-thalassemia TN disease with additional observations on hemoglobin CA-thalassemia TN disease. Blood (in press).

Cabannes, R., and J. Buhr. 1955. Une nouvelle fraction hémoglobinique humaine à migration électrophorétique plus rapide que l'hémoglobine A et moins rapide que l'hémoglobine H: Note préliminaire. Pédiatrie *10*: 888–889.

Cabannes, R., L. Sendra, and Dalaut. 1955. Nouvelle hémoglobine humaine héréditaire à migration plus rapide que l'hémoglobine normale. C. rend. soc. biol. *149*: 914 916.

CEPPELLINI, R. 1955. Discussion of paper by A. C. Allison. Cold Spring Harbor Sympos. Quant. Biol. *20*: 252–255.

CHERNOFF, A. I., V. MINNICH, and S. CHONGCHAREONSUK. 1954. Hemoglobin E, a hereditary abnormality of human hemoglobin. Science *120*: 605–606.

COHEN, F; W. W. ZUELZER; J. V. NEEL; and A. R. ROBINSON. Multiple inherited erythrocyte abnormalities in an American Negro family: hereditary spherocytosis, sickling, and thalassemia. Blood (in press).

CROSBY, W. H., and J. H. AKEROYD. 1952. The limit of hemoglobin synthesis in hereditary hemolytic anemia: Its relation to excretion of bile pigment. Am. J. Med. *13*: 273–283.

EDINGTON, G. M., and H. LEHMANN. 1954. Haemoglobin G. Lancet *2*: 173–174.

——— 1955. Expression of the sickle-cell gene in Africa. Brit. M. J. *2*: 1328.

FESSAS, P., and A. PAPASPYROU. 1957. New "fast" hemoglobin associated with thalassemia. Science *126*: 1119.

GERALD, P. S., and L. K. DIAMOND. 1958. The diagnosis of thalassemia trait by starch block electrophoresis of the hemoglobin. Blood *13*: 61–69.

GOUTTAS, A.; P. FESSAS; H. TSEVRENIS; and E. XEFTERI. 1955. Description d'une nouvelle variété d'anémie hémolytique congénitale: Étude hématologique, électrophorétique et génétique. Sang *26*: 911–919.

HALDANE, J. B. S. 1949. The rate of mutation of human genes. Proc. VIII Internat. Congr. Genet. Hereditas *35* (Suppl.): 267–273.

HARRIS, J. W. 1950. Studies on the destruction of red blood cells: VIII, Molecular orientation in sickle cell hemoglobin solutions. Proc. Soc. Exp. Biol., N.Y. *75*: 197–201.

HÖRLEIN, H., and G. WEBER. 1948. Ueber chronische familiäre Methämoglobinämie und eine neue Modifikation des Methämoglobins. Deut. med. Wschr. *73*: 476–478.

HUISMAN, T. H. J., J. H. P. JONXIS, and P. C. VAN DER SCHAAF. 1955. Amino-acid composition of four different kinds of human haemoglobin. Nature *175*: 902–903.

HUNT, J. A., and V. M. INGRAM. 1958. Allelomorphism and the chemical differences of the human haemoglobins A, S, and C. Nature *181*: 1062–1063.

INGRAM, V. M. 1957. Gene mutations in human haemoglobin: The chemical difference between normal and sickle cell haemoglobin. Nature *180*: 326–328.

——— 1959. Chemistry of the abnormal human haemoglobins. Brit. M. Bull. *15*: 27–32.

ITANO, H. A. 1951. A third abnormal hemoglobin associated with hereditary hemolytic anemia. Proc. Nat. Acad. Sc. U.S. *37*: 775–784.

——— 1953. Qualitative and quantitative control of adult hemoglobin synthesis—a multiple allele hypothesis. Am. J. Human Genet. *5*: 34–45.

——— 1955. Clinical states associated with alterations of the hemoglobin molecule. Arch. Int. M. *96*: 287–297.

ITANO, H. A., and J. V. NEEL. 1950. A new inherited abnormality of human hemoglobin. Proc. Nat. Acad. Sc. U.S. *36*: 613–617.

ITANO, H. A., W. R. BERGREN, and P. STURGEON. 1954. Identification of a fourth abnormal human hemoglobin. J. Am. Chem. Soc. *76*: 2278.

JACOB, G. F., and A. B. RAPER. 1958. Hereditary persistence of foetal haemoglobin production, and its interaction with the sickle-cell trait. Brit. J. Haemat. *4*: 138–149.

JAMES, G. W., and L. D. ABBOTT. 1955. Erythrocyte destruction in sickle-cell anemia: Simultaneous N^{15}-hemin and N^{15}-stercobilin studies. Proc. Soc. Exp. Biol., N.Y. *88*: 398–402.

JENSEN, W. N., E. B. PAGE, and D. L. RUCKNAGEL. 1955. Hemoglobin H: an inherited hemoglobin anomaly. Clin. Res. Proc. *3*: 93.

JOSEPHSON, A. M.; M. S. MASRI; L. SINGER; D. DWORKIN; and K. SINGER. 1958. Starch block electrophoretic studies of human hemoglobin solutions. II. Results in cord blood, thalassemia and other hematologic disorders: Comparison with Tiselius electrophoresis. Blood *13*: 543–551.

KAPLAN, E., W. W. ZUELZER, and J. V. NEEL. 1951. A new inherited abnormality of hemoglobin and its interaction with sickle cell hemoglobin. Blood *6*: 1240–1259.

KEITEL, H. G., and D. THOMPSON. 1956. The urinary concentration defect in sicklemia. Clin. Res. Proc. *4*: 7–8.

KEITEL, H. G., D. THOMPSON, and H. A. ITANO. 1956. Hyposthenuria in sickle cell anemia: a reversible renal defect. J. Clin. Invest. *35*: 998–1007.

KUNKEL, H. G., and G. WALLENIUS. 1955. New hemoglobin in normal adult blood. Science *122*: 288.

KUNKEL, H. G.; R. CEPPELLINI; U. MÜLLER-EBERHARD; and J. WOLF. 1957. Observations on the minor basic hemoglobin component in the blood of normal individuals and patients with thalassemia. J. Clin. Invest. *36*: 1615–1625.

KUNKEL, H. G., R. CEPPELLINI, and L. C. DUNN. 1958. A minor hemoglobin variant related to A_2 hemoglobin. Paper read at the meeting of the American Society of Hematology, April 26–27, 1958.

KUNZ, H. W.; E. L. PRATT; G. W. MELLIN; and M. W. CHEUNG. 1954. Impairment of urinary concentration in sickle cell anemia. Pediatrics *13*: 352–356.

LIVINGSTONE, F. B. 1958. The distribution of the sickle cell gene in Liberia. Am. J. Human Genet. *10*: 33–41.

LUAN ENG, L.-I. 1956. Penjelidikan hemoglobin patologik di Indonesia. Thesis, Universitas Indonesia di Djakarta. Pp. 154.

LUAN ENG, L.-I., and SADONO. 1958. Haemoglobin O (Bugenese X) in Sulawesi. Brit. M. J. *1*: 1461–1463.

MCCRORY, W. W., N. GOREN, and D. GORNFELD. 1953. Demonstration of impairment of urinary concentration ability or "Pitressin-resistance" in children with sickle-cell anemia. Am. J. Dis. Child. *86*: 512–513.

MCMASTER, J.; C. J. ZARAFONETIS; L. MOLTHAN; and W. A. STEIGER. 1956. Apparent renal defect in sicklemic individuals. Clin. Res. Proc. *4*: 81.

MINNICH, V.; S. NA-NAKORN; S. CHONGCHAREONSUK; and S. KOCHASENI. 1954. Mediterranean anemia: A study of thirty-two cases in Thailand. Blood *9*: 1–23.

MILLER, M. J., J. V. NEEL, and F. B. LIVINGSTONE. 1956. Distribution of parasites in the red cells of sickle-cell trait carriers infected with *Plasmodium falciparum*. Tr. R. Soc. Trop. M. Hyg., Lond. *50*: 294–296.

NA-NAKORN, S., and V. MINNICH. 1957. Studies on hemoglobin E. III. Homozygous hemoglobin E and variants of thalassemia and hemoglobin E: a family study. Blood *12*: 529–538.

NEEL, J. V. 1949. The inheritance of sickle cell anemia. Science *110*: 64–66.

——— 1951. The inheritance of the sickling phenomenon, with particular reference to sickle cell disease. Blood *6*: 389–412.

——— 1957. Human hemoglobin types: Their epidemiologic implications. N. England J. M. *256*: 161–171.

——— 1958. The genetic control of hemoglobin synthesis. Proc. VI Internat. Congr. Int. Soc. Hemat., pp. 665–672.

——— Genetic Aspects of Abnormal Hemoglobins. National Academy of Sciences–National Research Council Conference on Hemoglobin (in press, a).

——— Genetic Aspects of Abnormal Hemoglobins. C.I.O.M.S. Conference on Hemoglobin (in press, b).

NEEL, J. V., *et al.* 1953. A system of nomenclature for the varieties of human hemoglobin. Science *118*: 116–117.

NEEL, J. V., and W. N. VALENTINE. 1947. Further studies on the genetics of thalassemia. Genetics *32*: 38–63.

NEEL, J. V., I. C. WELLS, and H. A. ITANO. 1951. Familial differences in the proportion of abnormal hemoglobin present in the sickle cell trait. J. Clin. Invest. *30*: 1120–1124.

NEEL, J. V.; J. HIERNAUX; J. LINHARD; A. ROBINSON; W. W. ZUELZER; and F. B. LIVINGSTONE. 1956. Data on the occurrence of hemoglobin C and other abnormal hemoglobins in some African populations. Am. J. Human Genet. *8*: 138–150.

OLSEN, E. B.; K. OLSEN; F. B. LIVINGSTONE; W. W. ZUELZER; A. R. ROBINSON; and J. V. NEEL. Thalassemia in Liberia. Brit. M. J. (in press).

PAULING, L.; H. A. ITANO; S. J. SINGER; and I. C. WELLS. 1949. Sickle cell anemia, a molecular disease. Science *110*: 543–548.

RANNEY, H. M. 1954. Observations on the inheritance of sickle-cell hemoglobin and hemoglobin C. J. Clin. Invest. *33*: 1634–1641.

RAPER, A. B. 1956. Sickling in relation to morbidity from malaria and other diseases. Brit. M. J. *1*: 965–966.

RICH, A. 1952. Studies on the hemoglobin of Cooley's anemia and Cooley's trait. Proc. Nat. Acad. Sc. U.S. *38*: 187–196.

RIGAS, D. A., R. D. KOLER, and E. E. OSGOOD. 1955. New hemoglobin possessing a higher electrophoretic mobility than normal adult hemoglobin. Science *121*: 372.

ROBINSON, A. R.; W. W. ZUELZER; J. V. NEEL; F. B. LIVINGSTONE; and M. J. MILLER. 1956. Two "fast" hemoglobin components in Liberian blood samples. Blood *11*: 902–906.

RODMAN, T.; H. P. CLOSE; W. FRAIMON; R. CATHCART; and M. K. PURCELL. 1958. The oxygen dissociation curve in the common hemoglobinopathes. Clin. Res. Proc. *6*: 189–190.

SCHNEIDER, R. G., and M. E. HAGGARD. 1957. A new haemoglobin variant exhibiting anomalous electrophoretic behaviour. Nature *180*: 1486–1487.

SCHWARTZ, H., and T. H. SPAET. 1955. Hemoglobin G: A fifth abnormal hemoglobin. Clin. Res. Proc. *3*: 51–52.

SCHWARTZ, H. C.; T. H. SPAET; W. W. ZUELZER; J. V. NEEL; A. R. ROBINSON; and S. F. KAUFMAN. 1957. Combinations of hemoglobin G, hemoglobin S, and thalassemia occurring in one family. Blood *12*: 238–250.

SCOTT, R. B.; A. D. FERGUSON; M. E. JENKINS; and H. M. CLARK. 1955. Studies in sickle-cell anemia. VIII. Further observations on the clinical manifestations of sickle-cell anemia in children. Am. J. Dis. Child. *90*: 682–691.

SILVESTRONI, E., and I. BIANCO. 1952. Genetic aspects of sickle cell anemia and microdrepanocytic disease. Blood *7*: 429–435.

SINGER, K. 1955. Hereditary hemolytic disorders associated with abnormal hemoglobins. Am. J. Med. *18*: 633–652.

SINGER, K., A. I. CHERNOFF, and L. SINGER. 1951. Studies on abnormal hemoglobins. I. Their demonstration in sickle cell anemia and other hematologic disorders by means of alkali denaturation. Blood *6*: 413–428.

SINGER, K., and B. FISHER. 1952. Studies on abnormal hemoglobins. V. The distribution of type S (sickle cell) hemoglobin and type F (alkali resistant) hemoglobin within the red cell population in sickle cell anemia. Blood *7*: 1216–1226.

SINGER, K.; A. P. KRAUS; L. SINGER; H. M. RUBINSTEIN; and S. R. GOLDBERG. 1954. Studies on abnormal hemoglobins. X. A new syndrome: hemoglobin C-thalassemia disease. Blood *9*: 1032–1046.

SINGER, K., L. SINGER, and S. R. GOLDBERG. 1955. Studies on abnormal hemoglobins. XI. Sickle cell-thalassemia disease in the Negro: The significance of the S + A + F and S + A patterns obtained by hemoglobin analysis. Blood *10*: 405–415.

SMITH, E. W., and J. V. TORBERT. 1958. Study of two abnormal hemoglobins with evidence for a new genetic locus for hemoglobin formation. Bull. Johns Hopkins Hosp. *102*: 38–45.

STURGEON, P., and C. A. FINCH. 1957. Erythrokinetics in Cooley's anemia. Blood *12*: 64–73.

TERRY, D. W., A. G. MOTULSKY, and C. E. RATH. 1954. Homozygous hemoglobin C: A new hereditary hemolytic disease. N. England J. M. *251*: 365–373.

THOMAS, E. D., A. G. MOTULSKY, and D. H. WALTERS. 1955. Homozygous hemoglobin C disease: Report of a case with studies on the pathophysiology of neonatal formation of hemoglobin C. Am. J. Med. *18*: 832–838.

THORUP, O. A.; H. A. ITANO; M. WHEBY; and B. S. LEAVELL. 1956. Hemoglobin J. Science *123*: 889–890.

VANDEPITTE, J. Importance des hémoglobinoses au Congo Belge. C.I.O.M.S. Conference on Hemoglobin (in press).

VANDEPITTE, J. M.; W. W. ZUELZER; J. V. NEEL; and J. COLAERT. 1955. Evidence concerning the inadequacy of mutation as an explanation of the frequency of the sickle cell gene in the Belgian Congo. Blood *10*: 341–350.

VANDEPITTE, J., and J. DELAISSE. 1957. Sicklémie et paludisme. Ann. Soc. belge méd. trop. *37*: 703–736.

VELLA, F.; R. H. C. WELLS; J. A. M. AGER; and H. LEHMANN. 1958. A haemoglobinopathy involving haemoglobin H and a new (Q) haemoglobin. Brit. M. J. *1*: 752–755.

WENT, L. N., and J. E. MACIVER. 1958. An unusual type of hemoglobinopathy resembling sickle cell-thalassemia disease in a Jamaican family. Blood *13*: 559–568.

ZANNOS, L. 1953. Studies on the resistance of haemoglobin to alkali. Acta paediat., Upps. *42*: 305–312.

ZARAFONETIS, C. J. D.; W. A. STEIGER; L. MOLTHAN; J. MCMASTER; V. F. COLVILLE; and R. STAUFFENBERG. 1955. Hyposthenuria in sickle cell trait and sickle cell disease. Clin. Res. Proc. *3*: 29–30.

ZUELZER, W. W., and E. KAPLAN. 1954. Thalassemia-hemoglobin C disease—a new syndrome presumably due to the combination of the genes for thalassemia and hemoglobin C. Blood *9*: 1047–1054.

ZUELZER, W. W., J. V. NEEL, and A. R. ROBINSON. 1956. Abnormal hemoglobins. Chap. v *in* Progress in Hematology. New York: Grune & Stratton.

SOME FURTHER OBSERVATIONS ON ASSOCIATIONS BETWEEN BLOOD GROUPS AND DISEASE

J. A. Fraser Roberts[1]

THE EVIDENCE that there are associations between the ABO blood groups and certain diseases seems quite overwhelming, though I believe this is still doubted by some authorities. The strongest evidence is that relating to duodenal ulceration. If the published series are combined, the incidence of this disease in persons of group O is about 40 per cent higher than in members of the other groups. The total numbers now exceed ten thousand and χ^2 is well over two hundred for one degree of freedom. There is some evidence of heterogeneity, but the striking fact is that every series so far investigated shows an excess of group O. To begin with, the series came from the white races only, but now we have similar results for American negroes (Buckwalter *et al.*, 1957). And I believe that we shall soon hear of similar findings in other races as well. For cancer of the stomach the higher incidence in persons of group A as compared with those of groups O and B yields a χ^2 of over fifty. There is no evidence as yet to contradict the hypothesis that all the published series are homogeneous.

Doubts have sometimes been expressed about the comparability of the control series, but possible disturbances due to this cause are of the wrong order of magnitude. The only differences we can recognize with series of the size so far investigated are large. A relative incidence of 1.1 to 1.0 between the common groups O and A is about the limit, and even this needs numbers of the order of, say, six or seven thousand if we are to be reasonably sure of picking it up. Now all experience shows that differences between control series drawn from the same area are trivial in comparison. We can also make a second kind of comparison. Many of the series for duodenal ulceration and cancer of the stomach come from the same workers at the same centres, indeed from the same hospitals. If we look at the findings for these two diseases alone, the χ^2 is again two hundred or so. If at any centre the controls happen to be too high in O, this will magnify unduly the deviation for cancer of the stomach, but at the same time it will minimize the deviation for duodenal ulcer; and a similar deviation will occur if the controls happen to be rather too high in A.

If, then, we accept certain associations as proved beyond all reasonable doubt, the next question to ask is whether they are meaningful. Conceivably they might be real enough in a sense, but of little interest because they are secondary to stratifications in the population, racial or other, though the evidence against stratification seems very strong. In the allied secretor system, with the strong association between non-secretion and duodenal ulceration, Clarke and his colleagues (1956) have produced direct evidence against it; non-secretors are more susceptible than their own secretor sibs. The indirect evidence, however, is stronger and more general. It taxes credulity to imagine that the same stratifications could exist in so many different parts of the world; that, for example, in all the numerous centres investi-

[1]Clinical Genetics Research Unit (Medical Research Council), Institute of Child Health, The Hospital for Sick Children, London, England.

gated there were strains in the population characterized by a high frequency of gene *O* and at the same time undue susceptibility to duodenal ulcer. Furthermore, the facts we have do not indicate that, apart from racial mixture, populations are appreciably stratified for ABO frequencies. To give just one recent finding, Dawson (1958) analyses gene frequencies in relation to occupation in the County of Dublin and finds there is little evidence to suggest heterogeneity of ABO frequencies as between different occupational groups.

Another very strong point is the highly specific nature of all the ABO associations for which there are really convincing findings. They are all diseases of or associated with the upper part of the gastro-intestinal tract: namely, duodenal ulceration, gastric ulceration, cancer of the stomach, pernicious anaemia, and, less certainly, diabetes mellitus. The recent, truly remarkable results of Cameron (1958) pinpoint the same region of the body. He finds with tumours of the salivary glands 206 A's to 100 O's in the disease series as against 3,177 O's to 1,906 A's in a sample of the donor panel of Glasgow. This may well prove to be the most important association yet discovered. The incidence of salivary gland tumours in persons of group A is no less than about three and a half times greater in persons of group A as compared with those of group O. And, of course, as it has always seemed likeliest to me, if these ABO associations are in fact to be ascribed to some effect of substances A, B, and H, then presumably the salivary glands are just the places where one might expect the effect to be at its maximum. I am quite sure that Cameron's most valuable work will be quickly repeated at other centres. This is indeed desirable.

I have one small contribution to make in the way of preliminary results. Recently Professor Aird's team, with which I am very glad to be associated, has collected a series of 620 cases of cancer of the pancreas from a number of hospitals in Great Britain. The relative incidence of this disease in patients of blood group A is 1.24 against 1 in those of groups O and B. The χ^2 is the very modest one of 6.6 for 1 degree of freedom. In the ordinary way one would regard this as no more than an indication for further work, but in this particular instance it is interesting to see suggestive evidence emerging for yet another association with a disease of the gastro-intestinal tract.

We have tried to collect data on another relatively rare disease, cancer of the oesophagus; the number so far is 610. Once again there is some excess of group A, but this time the mean weighted relative incidence is no more than 1.12, and the difference from unity is well below the 5 per cent level of significanc. But, of course, the figure is equally compatible with the 1.2 to 1 found with cancer of the stomach. It is one more indication, if we needed it, of the very large numbers required in these studies. It could easily happen, if the figures were not co-operative, that we might collect a sample two or three times as large and still not know whether cancer of the oesophagus goes with cancer of the stomach or not. Further series will have to be collected.

I should now like to turn to another kind of result, namely, the discovery of really large associations in comparatively small series at single centres, associations large enough to give highly significant differences on quite small numbers. Of course, such associations have to be relatively very large or else they could not be discovered in this way. The first was Struthers' finding (1951) at Glasgow of a very large excess of group A in infants dying of or with bronchopneumonia. My colleagues, Carter and Heslop (1957), have recently repeated this work in London and find no evidence for any association. True the series are not quite comparable because one-third of Struthers' patients had died of the disease, whereas bronchopneumonia was only an associated finding in the London series. Nevertheless, the results seem to be

in very definite conflict. Now I am sure we should be making a great mistake if we were to say that Struthers was wrong and Carter and Heslop right, that is, if we accepted the negative result simply because it was negative. Conceivably both are right, and it would be unfortunate if further work were not carried out.

In the field of the association of blood groups and disease it seems inappropriate to talk of "confirmation." With the present excellence of grouping techniques and with an understanding of the precautions to be taken in choosing control series, as well as the fortunate stability of gene frequencies shown by such series, the problem is not one of confirmation or non-confirmation, but of combination of data. If we find heterogeneity, that too presents a problem for investigation.

A second remarkable association is Billington's (1956) very high excess of A in patients suffering from portal cirrhosis, but as far as I know this work has not yet been repeated elsewhere. At the moment the most instructive example seems to be that of Mayr and his colleagues (1956) who studied a series of more than six hundred patients with cerebral tumour. With all but one there was no apparent association, but in 123 patients with adenoma of the pituitary there was a relatively enormous excess of group O, and this appeared at all the hospitals included. Later Damon (1957) published a larger series from the Presbyterian Hospital in New York. His results were in conflict with those of Mayr, but there was still some excess of group O, both in whites and in negroes. We have tried to collect cases of this rare tumour and have managed, thanks to the collaboration of a number of hospitals in Great Britain, to secure 397. There is no evidence for any over-all association of any kind, but the figures from the different centres are suspiciously variable. Even with these small numbers the χ^2 for heterogeneity attains the 5 per cent level of significance. When the American and British results are combined, the χ^2 for an over-all association with O is of no more than borderline significance, but the heterogeneity is striking, χ^2 being significant beyond the 5 per cent point. I should also mention that in our series Manchester contributed the very peculiar result of twenty-two O's to six A's, and there was also a less striking but still rather notable excess of O's at one of the London hospitals.

It seems to me that there is a phenomenon to investigate here. Personally I cannot think of any explanation for such conflicting results at different centres. I hope, therefore, that others will go on adding to the numbers of adenomas of the pituitary studied. It may be that something of value and importance may emerge.

So far only disease incidences as a whole have been mentioned. Clearly, there is much scope for work on subdivisions—subdivisions by type, severity, and site of disease, by age, by sex, and so forth. This work is not easy: subdivision means smaller and smaller samples, and the total numbers needed necessarily become very large indeed. There is also the difficulty of securing comparable subdivisions at different centres in different countries. A beginning has been made. It seems very probable that in patients with cancer of the stomach the group frequencies differ according to the site of the tumour. I will mention only one other particular association, on which I am able to quote some findings not yet published. This concerns the stomal or anastomotic ulcer of the duodenum. In patients with this condition fresh ulcers continue to appear, and it may be regarded as the most severe form of the ulcer diathesis. Brown and his colleagues at Glasgow (1956) found that the number of patients with stomal ulcers was especially high in group O. A re-examination of our own data supported this finding. Dr. Richard Doll has now collected a much larger series of well over two hundred patients with stomal ulcers and has kindly allowed me to quote his findings. These are exactly in line with the previous results. There can be little or no doubt, therefore, that the incidence in persons of

group O of this particularly severe tendency to ulceration is about double that found in persons belonging to the other groups. Here, then, is another and very striking association, once again pinpointing the close kinship between ABO groupings and some of the diseases of the upper part of the gastro-intestinal tract.

When Fisher and Ford, more than twenty years ago, urged that a search be made for associations between blood groups and disease, I rather imagine that what they anticipated was not what has emerged with the ABO groups. They probably anticipated that many diseases, perhaps almost all, would show differences in incidence in the different genotypes or phenotypes, and that some or all of these differences might be small or very small. But what has emerged is relatively very large associations with a few highly specific disease entities, and, within the limits imposed by numbers, negative results for all the others that have been investigated. Of course, the widespread small association may exist, but the numbers needed to demonstrate it would of course need to be truly vast. At the moment, a difference in relative incidence of 5 per cent is almost beyond practicable limits. And, of course, we all know that a selective advantage of 5 per cent, or even of 2 or 1 per cent, may be of great importance from the point of view of natural selection. It may be, however, that practical needs may in the future supply data automatically. In the meantime I will mention just one fairly large-scale investigation. Through the Royal Medico-Psychological Association some fifty mental hospitals in Great Britain agreed to group their new admissions during 1957. The total number was about twenty thousand and so far forms have been coded and cards punched for about half. We have made a very brief preliminary analysis so as to make a report of some kind at this Congress. On a count of ten thousand, there is no indication of any difference between the figures for mental patients and those for the general population for the various areas. Nor does a broad grouping of main mental diseases show any significant variation. But, of course, the total number, and a much more detailed analysis, may yet bring something to light.

The general biological interest of the associations is considerable, particularly their bearing on balanced polymorphism, the neutral gene, and genetic drift. The elucidation of many aspects of these problems must, of course, depend on studies carried out with experimental animals and plants. Nevertheless, human genes have often been invoked, and they did appear to possess certain advantages. There is the clear evidence that some at least of the human polymorphisms are as old as the species; there is the advantage of what is effectively a randomly breeding population; there are the vast numbers blood grouped for practical purposes, leading to an immense body of knowledge on geographical variation. Unfortunately, the reasonable assumption that in a species under such close observation any selective advantages and disadvantages should be either fairly obvious, or at least not too difficult to discover, proved to be mistaken.

Dobzhansky, in the edition of his *Genetics and the Origin of Species* published in 1951, sums up one side of the argument very fairly: "It has been stated . . . that no two genotypes are likely to be exactly equal in adaptive value. Taken literally, this means that any genetic change must be either useful or harmful to its possessors, and that no adaptively neutral traits can exist. This is indeed the view espoused by Fisher . . . Ford . . . and their schools. Such a view leads, however, to difficulties when applied to many concrete situations." After mentioning the ABO and MN blood groups and P.T.C., he continues:

> It [polymorphism] is a very ancient condition, inherited possibly from the common ancestors of man and the apes. Fisher *et al.* infer that the polymorphisms in the blood groups, tasting phenyl-thio-carbamide, etc., must be balanced by heterozygotes possessing higher adaptive

values than the homozygotes. There is, however, absolutely no evidence that heterozygosis or homozygosis for most of the blood group genes, or for tasting phenyl-thio-carbamide, bring any advantages or disadvantages whatsoever to their possessors. It must be conceded that the absence of such evidence does not prove the converse proposition, that these traits are neutral. But to assume that the polymorphism is always adaptive and governed by selection means to take for granted what has thus far proved impossible to demonstrate. There is no compelling necessity to adopt such a course, because alternative possibilities certainly exist.

Dobzhansky goes on to mention equilibria maintained by opposing mutation rates, and also genetic drift, but he is careful to point out that there may be an interaction of genetic drift and natural selection.

With the Rhesus system, thanks to the brilliant work of Levine and his colleagues, it has been clear from the very outset that strong selective pressures must be involved. This is also true of the Kell system. When, however, selective advantages and disadvantages are not due, or apparently are not due, to antigenic reactions they may, as we now know, be very difficult to discover. They may be relatively small in magnitude and so not detectable in series of any ordinary size, but this possibility is hypothetical. They may, and this is not hypothetical, be highly specific, and even if they are large may not come to light unless the investigator happens to study that particular association out of all the hundreds he might look at. The idea that in human beings such associations should be obvious or easily detected proves a broken reed.

At this point I should like to mention one of the most striking examples of all, phenylthiocarbamide (P.T.C.). P.T.C. does not occur in nature, but is chemically allied to certain substances known to be goitrogenic. This alliance prompted an inspired guess by Harris, Kalmus, and Trotter (1949). They found that patients suffering from nodular goitre included 40 per cent of non-tasters, instead of the usual 30 per cent. Significance based on their relatively small numbers was little more than borderline, but the observations have now been repeated on a larger scale by Howel-Evans and Kitchin (1958) and the original result is fully confirmed. Once again we have a picture of extreme specificity; it is not goitre as a whole, or simple goitre as a whole, or toxic goitre as a whole, which is associated with non-tasting. It is only nodular goitre, whether toxic or non-toxic.

For some time the authropologists had been losing faith in the harmless inherited quirk, devoid of selective value. For a time they thought that genetics would supply them with substitutes. But even if harmless inherited quirks exist, how can their harmlessness be proved? Human genetics will doubtless go on providing examples of balanced polymorphism, but how can it produce convincing evidence for a neutral gene?

In discussions on balanced polymorphism, heterozygote advantage bulks very large, and this may indeed be the usual mechanism; it is certainly the simplest. But there are other and more complicated possibilities which may take a great deal of working out. With the Rhesus and Kell systems it is homozygosity of populations which confers the advantage. Then, to mention only one further example, there is Levine's original discovery that ABO incompatibility of mother and child protects very strongly against haemolytic disease, an observation which has since been very thoroughly confirmed. Levine (quoted by Race and Sanger, 1958) now finds that the same thing is true with Kell. Here, then, are selective pressures depending on two allelic systems simultaneously and affected by the gene frequencies of both. At present, the actual mechanisms must often be obscure, but we can at least say that the evidence for selective pressures is there.

The discovery that strong selective pressures are involved does not rob the blood group and other polymorphic systems of their value in anthropology, for there is every indication that, in general, changes in gene frequencies must be relatively slow. The contrast with the abnormal haemoglobins and with thalassaemia is striking. There is some direct evidence. Very large series, of the order of 100,000 or so, show no significant differences in age distribution of blood donors of the four ABO groups. Thus, there is no evidence at all of change over a generation and a half. The indirect evidence is, however, far stronger. The world blood-group distributions are quite incompatible with rapid change, and again and again while differences in gene frequencies can plausibly be explained in terms of migrations and intercrossings, they would be very difficult to account for on theories of adaptation. To quote a recent example, Dawson and Hackett (1958) found differences in ABO and Rhesus frequencies in the County of Dublin, and these fit very well with what is known historically about that region during the last thousand years.

I think we may hope that recent advances hold out the promise of very interesting work for the immediate future, both theoretically and practically. At the same time anthropologists, even if they have to add the extra dimension of adaptation, will undoubtedly continue to find human polymorphic systems of increasing value in tracing the relationships, migrations, and intercrossings of mankind. It may prove ultimately that the addition of the extra dimension, far from being a disadvantage, is what is required to complete an ordered synthesis.

REFERENCES

Billington, B. P. 1956. A note on the distribution of ABO blood groups in bronchiectasis and portal cirrhosis. Australas. Ann. M. *5*: 20–22.

Brown, D. A. P., A. G. Melrose, and J. Wallace. 1956. The blood groups in peptic ulceration. Brit. M. J. *2*: 135–138.

Buckwalter, J. A.; J. H. Turner; L. Raterman; R. T. Tidrick; and L. A. Knowler. 1957. Ethnologic aspects of the ABO blood groups disease associations. J. Am. M. Ass. *165*: 327–329.

Cameron, J. M. 1958. Blood-groups in tumours of salivary tissue. Lancet *1*: 239–240.

Carter, C., and B. Heslop. 1957. ABO blood groups and bronchopneumonia in children. Brit. J. Prev. Social M. *11*: 214–216.

Clarke, C. A.; J. W. Edwards; D. R. W. Haddock; A. W. Howel-Evans; R. B. McConnell; and P. M. Sheppard. 1956. I, ABO blood groups in duodenal ulcer; II, Secretor character in duodenal ulcer. Brit. M. J. *2*: 725–731.

Damon, A. 1957. Blood groups in pituitary adenoma—"suspected correlation" reexamined. Science *126*: 452–453.

Dawson, G. W. P. 1958. The blood group frequencies in some occupational groups in county Dublin. Ann. Human Genet., Lond. *22*: 315–322.

Dawson, G. W. P., and W. E. R. Hackett. 1958. A blood group survey of the county and city of Dublin. Ann. Human Genet., Lond. *22*: 97–110.

Dobzhansky, T. G. 1951. Genetics and the Origin of Species. 3rd revised edition. New York: Columbia Univ. Press.

Harris, H., H. Kalmus, and W. R. Trotter. 1949. Taste sensitivity to phenylthiourea in goitre and diabetes. Lancet *2*: 1038–1039.

Howel-Evans, A. W., and F. D. Kitchin. 1958. Taste threshold for P.T.C. in thyroid disease. Heredity *12* (pt. 1): 144.

Mayr, E.; L. K. Diamond; R. P. Levine; and M. Mayr. 1956. Suspected correlation between blood-group frequency and pituitary adenomas. Science *124*: 932–934.

Race, R. R., and R. Sanger. 1958. Blood Groups in Man. 3rd edition. Oxford: Blackwell.

Struthers, D. 1951. ABO groups of infants and children dying in the west of Scotland (1949–1951). Brit. J. Social M. *5*: 223–228.

SYMPOSIUM

CYTOGENETICS AND PLANT BREEDING

1. John Unrau. Cytogenetics and Wheat-breeding
2. H. Kihara. Fertility and Morphological Variation in the Substitution and Restoration Backcrosses of the Hybrids, *Triticum vulgare* × *Aegilops caudata*
3. Donald F. Jones. Basic Research in Plant and Animal Improvement
4. C. Syrach Larsen. Genetics in Silviculture
5. M. S. Navashin. Cell Size and Development of Characters (paper not published)

CYTOGENETICS AND WHEAT-BREEDING

John Unrau[1]

THE RELATION of cytogenetics to wheat-breeding is obvious to all who believe that the chromosomes with their genes determine the mode of inheritance and also, by interacting with the environment, the expression of the characters of an organism. It is evident that if we accept the gene-chromosome mechanism as the basis of heritability of variation and likeness we should also accept as axiomatic that improvement through breeding can most effectively be realized the better this mechanism is understood. We must, in fact, go farther and conclude that, apart from gene mutations, it is through new combinations or arrangements of genes on the chromosomes that all improvements through breeding have come about. This is so regardless of whether or not the breeder believed in and understood the controlling mechanism. Consequently, the wheat-breeder on this basis must aim at manipulating, through the various techniques available, this hereditary mechanism in such a way as to produce better varieties, and ultimately more wheat per unit area seeded. That is, he applies the principles of genetics and cytology to the breeding of improved varieties of wheat. It serves a useful purpose to attempt an appraisal of the effectiveness of wheat-breeding programs, at least of those conducted with cytogenetic principles in mind. Many excellent examples cannot, of course, be considered in such an appraisal. Indeed, they would be beyond the scope of this paper; so the very brief evaluation will be restricted to results obtained in Sweden and Canada since the situations in these two countries are generally applicable elsewhere.

In Canada the value of wheat-breeding was recently appraised by Goulden (1956). He estimates that in the spring-wheat area of Canada, Thatcher and other new rust-resistant varieties have yielded 100 million bushels more annually than the famous Marquis variety. This improvement, however, represents mainly the increased production brought about by the higher yielding ability of the new varieties. Equally important is the earlier date at which they mature, enabling production in more northerly areas and safeguarding against damage by fall frosts. Even more dramatic is the improvement that has resulted from the resistance to rust of the new varieties. Rust resistance not only prevents damage to the resistant varieties in the threatened area but also reduces the danger that rust will spread to areas where nonresistant varieties are grown. Again, improvement is reflected not only in increases in total yield but also in the quality of the crop.

To these examples of improvement could be added others involving characteristics, perhaps important only locally, but contributing to stabilizing, safeguarding, and increasing total production.

In Sweden the situation is different only insofar as problems, and consequently objectives of breeding programs, are different. Müntzing (1950) has made a very helpful and enlightening review of the practical benefits from plant-breeding in Sweden. Yield of winter wheat has been increased 25 per cent by new varieties, representing additional earnings of twenty-five million kroner annually. Spring-wheat yields have been increased by 5 per cent, adding five million kroner annually.

[1]Professor of Plant Science, University of Alberta, Edmonton, Alta., Canada.

Again, it is clear that these increases are only part of the picture. Müntzing points out that, in fact, the increase in yield of spring wheat does not present a fair picture since through better adapted varieties production is now possible on poorer land. The aspect which Müntzing failed to stress but of which we in Canada are painfully aware is that the Swedish breeders have so improved the baking quality of their wheats that Canadian wheat is no longer required to strengthen Swedish flour. During the whole period reviewed (1866–1948) the Swedish government spent a total of about three million kroner on all plant-breeding.

What is true for these two countries is undoubtedly true for all wheat-producing countries in which breeding based on cytogenetic principles has been pursued. The investment has been small; the returns have been greater certainly than from most government-sponsored activities.

Early Work on Wheat Chromosomes

Genome Analysis

Cytologists were attracted quite early to wheat and its relatives, and one of their first contributions to wheat-breeders was a fairly concise (if oversimplified) concept of the genomes present in different species of wheat. The relation of different groups (as indicated by extent and intensity of chromosome-pairing) in hybrids confirmed the natural classification of wheat and established the phylogenetic relationships into diploid, tetraploid, and hexaploid groups to which for convenience the formulae AA, AABB, AAGG, and AABBDD, respectively, were assigned. Obviously, relations of homology or non-homology in hybrids with common genomes are not so discrete as the formulae would indicate. Indeed, those researchers mostly concerned with these investigations have clearly recognized this (Kihara, 1924; Thompson, 1925). The genome concept, while obviously an oversimplification of the natural and minute relations, has, however, greatly aided the wheat-breeder in planning his programs. Present and future work will be based to a large extent on genome relations, especially on those of forms related to wheat.

Relation of Chromosome Behavior in Triploid and Pentaploid Hybrids to Wheat-Breeding

It was fortunate that the study of chromosome behavior in triploid and pentaploid hybrids paralleled attempts by wheat-breeders to improve common wheat through the incorporation of characteristics mainly from the tetraploid species. Cytogenetic researches by Kihara (1924), Sax (1923), Thompson (1926, 1931), and others clearly indicated the cause of sterility in interspecific hybrids; the reasons for the paucity of plants with intermediate chromosome numbers; and the problems to be overcome in developing agronomically suitable common-wheat types having the desired characteristic from the tetraploid parent.

When reviewing the literature of the period from the 1920's to the early 30's, one is impressed by the apparent urgency with which both cytogenetic and breeding investigations of interspecific wheat hybrids were pursued on this continent. The resistance in available common-wheat varieties was ineffective against newer, more virulent races of stem rust. These races caused such serious epidemics that losses threatened the production of hard red spring wheat in large areas of the United States and Western Canada. Crosses of common wheats with resistant tetraploids (durums and emmers) were being intensively studied by cytogeneticists and plant-breeders; their progress (or lack of it) was watched with anxiety by administrators

who were responsible for financing research. There are the excellent studies of Sax (1923) and Sax and Gaines (1924) who concluded that in their material all stabilized, true-breeding, 28-chromosome forms had the distinguishing characteristics of the tetraploids, while the 42-chromosome forms had *vulgare* characteristics. Moreover, there had been complete failure to incorporate in these 42-chromosome forms the rust resistance of the tetraploids with the agronomic and quality characteristics of the bread wheats. Their conclusion, consequently, was that success in developing such derivatives from these crosses was highly improbable and they pointed out that up to that time, 1924, no new, commercially suitable variety had been obtained from these programs. But other cytogeneticists and wheat-breeders were getting results which, while not contradictory to those of Sax and Gaines, proved that an improbability is far from being an impossibility. The studies of Thompson and coworkers (1925, 1927, 1935) showed that not all of the distinguishing characters of *T. vulgare* were controlled by the D-genome; that individuals with euploid chromosome numbers were the only stable combinations; and that 42-chromosome types having the rust resistance of the tetraploids and agronomic characteristics of *vulgare* were rarely obtained from the pentaploid hybrids. The solution obviously lay in growing large populations, as the breeding work of Hayes *et al.* (1920), Harrington and Smith (1929), Goulden (1929), and McFadden (1930) proved. Even so, by the late 1920's there was no new resistant variety and the epidemic outbreaks of rust were, if anything, becoming more serious. We can, I believe, understand and appreciate the situation when we remember that the late E. S. McFadden (Babcock, 1940) gave the name "Hope" to a line obtained from an emmer × *vulgare* cross, having rust resistance and predominantly bread-wheat characters. This variety as well as Marquillo, a derivative of the cross Marquis × *Iumillo durum* made at the University of Minnesota, and a *vulgare*-like hybrid obtained at the Dominion of Canada Rust Research Laboratory, Winnipeg, were all somewhat unsatisfactory as bread wheats, but they formed the advance base from which success was achieved. We find them as parents of the "Greats" in the annals of rust-resistant spring wheats in North America: Thatcher, Renown, Regent, Apex, Rival, Mida, etc. And cytogenetic research was directly and indirectly a contributing factor in this development. Results from cytogenetic studies indicated the need for growing large populations to make the improbable possible; they also demonstrated the impossibility of obtaining true-breeding viable combinations with chromosome numbers other than 28 or 42.

These are not the only examples of genetic transfer of characters from species to species in wheat. Allard and Shands (1954) were successful in transferring the stem-rust and mildew resistance of *Triticum timopheevi* to common wheat. This was achieved by successively backcrossing resistant selections from segregating, backcross-derived populations to common wheat. Eventually plants were obtained that were cytologically stable and morphologically *vulgare*-like. Recently, also, Ohlendorf (1957) in crosses of *T. timopheevi* with a number of varieties of common wheat was able to extract fairly stable lines with resistance to mildew, leaf rust, and stripe rust from the *timopheevi* parent. The mechanism of character transfer in relation to cytogenetic and breeding behavior is clearly indicated in this work. Thus, (*a*) if the transfer occurred as a result of gene exchange between homologous chromosomes or chromosome segments, no cytogenetic or breeding complications will be encountered; (*b*) if it resulted from translocation involving non-homologous segments, the *timopheevi* segment may cause pairing difficulties and also lead to complications in the breeding program; (*c*) if the transfer resulted from the sub-

stitution of a whole chromosome, the future program depends on whether the transferred chromosome carries genes responsible for undesirable traits. If it does not, the whole chromosome can be carried along intact (with appropriate selection procedure) in future breeding programs. If undesirable traits are associated with it, additional and different treatment of the material must be undertaken before it can be utilized effectively in a breeding program. There are undoubtedly many unexplored possibilities of transferring not only disease resistance, but also characters of agronomic importance from the primitive to the common wheats (Fukasawa, 1949). This has been pointed out in a number of papers by Zhebrak (1944) who studied various synthetic amphiploids obtained from crosses of different *Triticum* species. Other workers, likewise, have been impressed with the agronomically promising segregates that can be obtained from crosses involving various amphidiploids. Thus, Riley and Chapman (personal communication), in a program involving the transfer of mildew resistance of *T. persicum* to *T. vulgare*, have obtained some selections which agronomically appear very promising from the cross (amphidiploid *T. aegilopoides* $\times$ *T. persicum*) $\times$ *T. vulgare*.

Intergeneric Hybrids and Derivatives

The possibility of introducing valuable characters into wheat from related genera has, through the years, been irresistibly fascinating. Certainly, published reports of work done are far too numerous and the scope is too varied to be reviewed in this paper. At best one can arbitrarily choose examples that illustrate how cytogenetic research, past and present, continues to contribute to wheat-breeding.

Hybridization has been successful between various species of *Triticum* and species of the genera *Secale*, *Aegilops*, *Haynaldia*, *Agropyron*, and *Elymus*. There are reports of hybridization with *Hordeum*, but repetition of these does not necessarily guarantee their authenticity. While considerable cytogenetic and breeding work has already been done with the many hybrids made, the true phylogenetic relations in these genera are just being established. Appropriate methods of realizing some of the practical possibilities of the various hybrid materials also are now being developed.

Wheat-Rye Hybrids and Amphiploids

Hybrids between wheat and rye have been known since before the turn of the century. Studies of natural and of artificially produced hybrids and amphiploids have been made by many European, North American, and Japanese workers (Bleier, 1928; Jesenko, 1913; Kagawa, 1939; Lebedeff, 1934; Ledingham and Thompson, 1938; Leighty and Sando, 1928; Lewitzky and Benetzkaja, 1932; Meister, 1921; Oehler, 1931; O'Mara, 1953; Riley and Chapman, 1957; Tschermak, 1910, 1931; Von Berg, Heinz, and Oehler, 1938). Although we cannot attempt to evaluate the many varied interpretations, the evidence is now conclusive that there is no true homology between any chromosome of rye and any of wheat (Sax and Gaines, 1924). It has also been established repeatedly that fertile forms, with various degrees of expression of characters of the parents, may be obtained from backcrossing, and that amphiploids can be obtained from natural or artificial doubling of the chromosomes. Cytogenetic research has shown that there is virtually no possibility of establishing hybrid-derived forms from which genes governing specific desirable characters of the rye parent can be incorporated into wheat by conventional breeding procedures.

Many investigators are sceptical of the practical value of the *Triticales*. As a group, these amphiploids have so far been disappointing. They do not have winter hardiness, they are low in yield, and most important, it is difficult to find a use for the grain which at best appears to be of indeterminate quality. It is too early even now, however, to reject entirely all *Triticales* as such. In fact, in a recent article, Pissarev (1957) reports that *Triticales* produced from carefully selected wheat and rye parents, under adverse conditions of the non-black soil regions of Russia, produce grain of higher protein content and also better bread-baking quality than wheat grown under these conditions.

Perhaps the greatest potential value of the *Triticales* lies in their use as source material for making chromosome substitutions and additions. Additions of single rye chromosomes to wheat have been studied by a number of investigators, notably Florell (1931), O'Mara (1953), and, more recently, Chapman and Riley (1955) at Cambridge. It is too early to ascertain whether any of the many possible forms obtainable will be useful directly, though some look attractive phenotypically.

Wheat-*Aegilops* Hybrids

The close phylogenetic relation of *Triticum* and *Aegilops* is indicated to some extent by the many hybrids that have been obtained between various members of the two genera. The evidence, based on synthsized species obtained by McFadden and Sears (1944) and Kihara (1944), working independently, conclusively indicates that the D-genome of the hexaploid wheats was contributed by *Aegilops squarrosa*. Recent work suggests that the B-genome also has been contributed by *Aegilops* (Sarkar and Stebbins, 1956; Sears, 1956b).

Characters of *Aegilops* that would be of great value if they could be incorporated into wheat are resistance to stem, leaf, and stripe rusts, resistance to insect damage, winter hardiness, and others listed by McFadden and Sears (1947).

Genomes of *Aegilops* have been combined directly with the hexaploid wheats or through the use of bridging crosses. The latter involve mainly amphiploids of the tetraploid wheats and diploid *Aegilops* crossed with the hexaploids. Such crosses are usually fertile, and they also bring together single genomes of *Aegilops* with the D-genome thus providing maximum opportunity for gene-exchange, presumably through occasional pairing of non-homologous chromosomes. Successful transfer of characters to hexaploids has been achieved, however, only by two widely different procedures reflecting the two different situations in the relations of the parental forms concerned.

Coffey (1956) recently was successful in transferring the stem-rust resistance of *Ae. speltoides* to hexaploid wheat. The amphiploid *T. dicoccoides* × *Ae. speltoides* was crossed with *T. aestivum* and a resistant derivative of this was crossed with natural and synthetic *T. spelta*. The transfer was apparently accomplished by direct gene exchange involving homology of the *speltoides* chromosome, carrying the resistance, with a chromosome of the wheat complement. Chromosome homology between *Ae. speltoides* and the B- or A-genome can be inferred not only from meiotic pairing but also from breeding behavior of amphiploids involving this species and various tetraploids (Sears, 1956b).

The transfer of the leaf-rust resistance from *Ae. umbellulata,* a species which apparently has no homology with any chromosomes of hexaploid wheat, was accomplished by Sears (1956a) using a different and completely new procedure. The chromosome carrying resistance was first added to wheat, and subsequently by

irradiation, a segment carrying the gene giving resistance was inserted in a wheat chromosome.

A great deal of interest and work has centered around what are commonly called *Aegilotricums* (Blaringhem, 1930; Kihara and Katayama, 1931; Oehler, 1936; Sorokina, 1937; Tschermak and Bleier, 1926). These are for the most part naturally occurring or artificially produced amphiploids involving various species of wheat and *Aegilops*. Although they have valuable characters, so far nothing of practical value has been obtained from them, undoubtedly because as such these amphiploids are unusable. To date, apparently, addition or substitution series from any of this material have not been established, and such lines probably are the only means whereby character transfers to common wheat can be made.

Wheat-*Haynaldia* Hybrids

Relatively few studies of crosses involving wheat and *Haynaldia villosa* have been made. Studies by Kostoff (1937) showed that probably no homology exists between *Haynaldia* and wheat chromosomes. Although McFadden and Sears (1947) concluded that pairing could occasionally take place, Hyde (1953) encountered no difficulties due to pairing when establishing wheat lines with individual chromosome additions from *Haynaldia villosa*.

Practical interest in *Haynaldia* arose mainly because of its resistance to disease. As for other crosses with forms having no homology with wheat, no transfer of characters has been obtained by ordinary breeding methods. Addition lines in this case do not appear too promising for transfer of stem-rust resistance, because either the resistance of *Haynaldia* is not expressed in the presence of the hexaploid wheat complement or the gamete functional in forming the original amphiploid did not carry the gene for rust resistance. *Haynaldia villosa* being cross-pollinated is highly heterozygous.

Wheat × *Agropyron* Hybrids

Almost unlimited benefits were envisaged by the extreme optimists from these hybrids when they were first being made and studied. Wheat-breeders saw the possibility of obtaining disease resistance, drought resistance, increased winter hardiness, and, of course, perennial wheat. Forage-breeders were no less enthusiastic. Their hope was a forage grass with a large seed which would overcome difficulties of establishing stands. None of these hopes has so far been realized.

Only a few crosses will be directly referred to here, since as early as 1942 Smith (1942) found that probably at least thirty different species of *Agropyron* had been used in crosses with wheat. The majority of successful crosses were with the so-called couch group, mainly *A. elongatum*, *A. intermedium*, and *A. tricophorum*. Both tetraploid and hexaploid wheats were used successfully.

With the possible exception of Kostoff (1941), most investigators attributed the high incidence of bivalent formation in these crosses to true homology of one (or possibly two) genomes of *Agropyron* with wheat (for example, Wakar, 1935; Peto, 1936; Matsumura, 1951). Because, in addition, there existed a fair degree of self-fertility in some, and good backcross fertility in most combinations, the hope of transferring desirable traits from *Agropyron* to wheat and vice versa appeared to be well founded. But breeding results have been disappointing. Work of Stebbins and Pun (1953) has clearly indicated the cytogenetic basis of the failure. Using the *Secale* genome as an analyzer, these workers proved that the pairing observed in F_1

was in fact completely attributable to autosyndesis of the highly autopolyploid *Agropyrons* that were used in the crosses. It is consequently highly improbable that any transfer of *Agropyron* characters has occurred through the normal process of gene exchange based on chromosomal homology. Most of the so-called promising stable wheatlike derivatives are octoploids having a complete *Agropyron* genome added to the wheat complement. Agronomically they are insufficiently wheatlike to be satisfactory. When backcrossing to wheat is undertaken, it is usually found (Marshall and Schmidt, 1954; Ohlendorf, 1955) that plants with the desired character from *Agropyron* also retain *Agropyron* chromosomes or parts of chromosomes, and so far this has invariably resulted in difficulties and irregularities of breeding behavior.

In the light of this, it is difficult to explain the claims by some investigators (Savchenko-Belsky, 1948; Tsitsin, 1946) that perennial wheats, and also wheats with greater winter hardiness, have been obtained in the progenies of wheat-*Agropyron* crosses. It is questionable that the so-called perennial wheats are actually to be classed as wheats. Generally, it has been found (White, 1940) that the perennial habit was also associated with grasslike characters. With respect to superior winter hardiness of some derivatives the simplest explanation is that they represent new combinations of wheat genes obtained from the different winter-wheat varieties used in the breeding programs after the hybrids were obtained.

These amphiploids can, however, be of value for transferring genes to wheat chromosomes by the use of X-irradiation. Such a transfer involving a gene for stem-rust resistance has been recently reported by Elliott (1957).

Hybrids with *Elymus*

To date only a few workers have reported success in obtaining crosses between wheat and *Elymus* (Pissarev and Vinogradova, 1944; Ragulin, 1947). Sterility in hybrids is high or even complete with no apparent chromosome homology, and practical utilization probably could only result from artificial transfer of chromatin by irradiation. *Elymus* has many characteristics that would be valuable if they could be transferred to wheat and efforts to do so will likely be continued.

Aneuploids and Related Types

Undoubtedly, from the standpoint of wheat genetics and breeding investigations, the development of the whole chromosome-deficient aneuploids by Sears (1939, 1944) and of various modified types by him and other workers represents the most important contribution of cytogenetics to wheat-breeding. The monosomics and nullisomics in Chinese Spring permitted the association with specific chromosomes and the nature of a number of genes through modification of the character controlled (Sears, 1944). But by far the greatest value has been, and will continue to be, the various manipulations with the aneuploids whereby chromosomes and parts of chromosomes can be studied for gene content, and whereby chromosomes from various sources can be systematically and deliberately combined.

The various steps involved in developing chromosome-deficient series in other varieties and in substituting individual chromosomes have been described by Sears (1953b) and Unrau *et al.* (Unrau, 1950; Unrau *et al.*, 1956). The procedures involved are not complex, but they are exacting, and selection and use of parents are based at every step on their chromosomal constitution.

The genetics of a number of characters has already been studied with great precision using aneuploid analysis. To date genes for stem-rust resistance in nine varieties have been associated with nine different chromosomes, as illustrated in Table I.

TABLE I

ASSOCIATION OF GENES FOR STEM-RUST RESISTANCE WITH SPECIFIC CHROMOSOMES IN RESISTANT VARIETIES OR LINES

Variety or line	Chromosome carrying gene(s) for resistance									Authority
	III	VI	VIII	X	XIII	XVI	XVII	XIX	XX	
Timstein	—	—	—	2	—	—	—	—	—	Sears and Rodenhiser (1948)
Hope	—	—	1	—	—	—	1	—	—	Sears *et al.* (1957)
Thatcher	1	—	—	—	1	—	—	1	—	Sears *et al.* (1957)
McMurachy	—	—	—	—	—	—	—	—	1	McGinnis (personal)
Red Egyptian	—	1	—	—	1	—	—	—	1	Sears *et al.*
Redman	1	—	1	—	1	—	—	—	—	McGinnis (personal)
Kenya Farmer	—	—	1	—	1	1	—	—	—	Knott (personal)
CI 12633	—	—	—	—	2	—	—	—	—	Nyquist(1957)
Kentana	—	—	—	—	—	—	—	—	—	Wiggin (1955)
Synth. Thatcher*	1	—	—	2	1	—	—	1	1	Kuspira and Unrau (unpublished)
Synth. Lemhi*	—	—	—	2	—	—	—	—	1	Kuspira and Unrau (unpublished)

*In these lines chromosomes X and XX have been substituted with the homologous chromosomes from the varieties Timstein and McMurachy respectively.

Already this information has cleared up many of the puzzling, and seemingly contradictory, results obtained in breeding and genetic studies involving stem-rust reaction. But more important, this information can be used for deliberately combining chromosomes of known genetic constitution. This use is illustrated by the line Thatcher (2 X Timstein, 2 XX McMurachy) developed at the University of Alberta (Kuspira and Unrau, unpublished) which in addition to the resistance of Thatcher, also has the resistance of Timstein and McMurachy, that is, a total of six genes for resistance.

Similarly, genes for reaction to other diseases and insects are being studied (Heyne and Livers, 1953), although so far sufficient information has not been available to permit a systematic program of combining chromosomes.

The aneuploids, and substitution lines derived from them, show promise as a means of indicating the location, the possible number, and the interaction of genes controlling characters having quantitative expression. Kuspira and Unrau (1957) found statistically highly significant modifications of yield, earliness, lodging resistance, height, and other characters, associated with different individual chromosomes of Thatcher, Hope, and Timstein substituted for their respective homologues in lines of Chinese Spring.

The effects of homologous chromosomes from the different donor varieties sometimes were similar and sometimes quite opposite, and in some instances the presence of allelic systems was indicated. It is conceivable that aneuploids will become extremely valuable for making specific modifications in a certain character of a variety by certain specific chromosome substitutions.

Aneuploids involving additions and substitutions of alien chromosomes or parts of chromosomes may be of value directly, or as sources for gene transfer by irradiation. In the simplest, ideal situation the addition or substitution chromosome may

carry the gene or genes for the characters to be transferred without alien genes having deleterious effects. Limited observations by Riley and Chapman (personal communication) and Jenkins (1951) indicate that certain rye-chromosome substitution lines are quite attractive, with the alien chromosome apparently compensating quite effectively for the wheat chromosome that was replaced.

If addition or substitution lines are not satisfactory as such, critical lines can be treated with X-ray, as was done by Sears (1956), to transfer leaf-rust resistance from *Aegilops umbellulata* to wheat and by Elliott (1957) to obtain stem-rust resistance from *Agropyron*. These are some of the possibilities cytogenetic studies with aneuploids can contribute to wheat-breeding. More are being discovered as more work is done with them. Quite recently, Okamoto (1957) and Riley and Chapman (personal communication) have discovered that the absence of a specific wheat chromosome greatly increases intensity and extent of pairing so that even non-homologous chromosomes are presumably involved. Speculation as to the possibilities this phenomenon offers would be premature at this stage, but utilization of wide crosses might conceivably be greatly simplified. The use of aneuploids and their derivatives represents a deliberate attempt at directing the evolution of wheat to more useful types by systematic chromosome-building. So much has been achieved in a short time that one cannot help being optimistic about the future. It must further be pointed out that many steps in the breeding program are based on selection made by cytological observations with the microscope, making special selection nurseries unnecessary. Thus, in combining chromosomes carrying rust resistance, no rust nurseries are required during the process of substituting and combining chromosomes.

Special Methods and Techniques

Doubling of Chromosomes

Colchicine and compounds with similar action have been tremendously valuable in producing fertile amphiploids of the many cross-combinations normally resulting in sterile F_1's. Undoubtedly, early in the history of its use (Blakeslee and Avery, 1937; Dustin, 1934; Eigsti and Dustin, 1955; Nebel and Ruttle, 1938) there was too much optimism regarding the practical value of all "doubled plants." Chromosome-doubling is, however, a necessary step in many present-day programs.

Irradiation

Many efforts are presently being made to improve crops, including wheat, by irradiation. Opinions as to the possibilities vary. So far as disease resistance is concerned, there apparently has been some success. Ausemus *et al.* (1955) report the production of a mutant in Lee similar to the control but with resistance to 15B of stem rust. Jenkins (1957) obtained a mutant in the *durum* variety Stewart, resistant to race 15B of stem rust, but in this instance other plant characters were also affected. Konzak *et al.* (1956) obtained a mutant resistant to stripe rust by means of thermal neutron treatment of the variety Gabo. There may be other examples since many investigators are using radiations as a part of breeding programs.

Possibly the most interesting use of irradiation is to transfer to the wheat complement chromatin carrying desirable genes from alien chromosomes. The limitation is in setting up the experiments in such a way that adequate cytogenetic studies may coincide with effective methods of selection.

Embryo Culture

Two techniques are of importance in cytogenetic and breeding work. Studies by Pissarev and Vinogradova (1944) and others have shown that crossability of distantly related forms is increased if the embryo of the plant to be used as male is cultured in the endosperm of the female. There are many valuable forms related to wheat that have not yet been crossed with wheat. The use of this method might lead to success in attempting the more difficult combinations.

The second technique is that of culturing young excised hybrid embryos on culture media. Again, this has given success in some combinations that ordinarily produced no viable hybrid seed. Both of these aspects hold promise of enabling cytogeneticists and wheat-breeders to make new, interesting, and probably valuable combinations with relatively distantly related species.

Summary

(1) An evaluation of the practical returns through increased production that have resulted from wheat-breeding based on cytogenetic principles is given for Sweden and Canada.

(2) Studies of chromosome numbers, karyotype, and homology have been and continue to be of great value in establishing the natural relations of wheat and its relatives. The wheat-breeder can be guided by these in choice of parents and type of program when breeding for certain characters.

(3) Cytogenetic studies provided basic information on the causes of sterility and difficulties of combining characteristics in interspecific crosses. This gave the practical breeder an understanding of the difficulty of the problem of obtaining rust resistance in good bread wheats and enabled him to enlarge his program accordingly.

(4) Cytogenetic studies have indicated the reasons for failure in most of the intergeneric hybrids with wheat. Many of the amphiploids obtained cannot be utilized directly, but may be valuable sources of useful addition or substitution lines.

(5) Aneuploids offer great possibilities to the breeder since through them the genetics of qualitative as well as quantitative characters can be studied with more accuracy. Through various manipulations, based on cytogenetic selection of parental types with certain chromosomal constitutions, plants with chromosomes having known gene content can be systematically built up. Substitutions involving partial or whole alien chromosomes offer possibilities of introducing new genes into the wheat complement.

(6) Colchicine, irradiation, and embryo-culture techniques have become indispensable to the cytogeneticist and wheat-breeder at certain stages of some programs.

References

Allard, R. W., and R. G. Shands. 1954. Inheritance of resistance to stem rust and powdery mildew in cytologically stable spring wheats derived from *Triticum timopheevi*. Phytopathology *44*: 266–274.

Ausemus, E. R., K. J. Hsu, and D. W. Sunderman. 1955. Resistance to stem rust race 15B induced by ionizing radiations. Agron. Abst. *47*: 48.

Babcock, E. B. 1940. The chronology of Hope wheat. J. Hered. *31*: 132–133.

BLAKESLEE, A. F., and A. G. AVERY. 1937. Methods of inducing doubling of chromosomes in plants by treatment with Colchicine. J. Hered. *28*: 393–411.
BLARINGHEM, L. 1930. Sur l'hérédité du sexe chez l'Ancolie (*Aquilegia vulgaris* L.). C. rend. Acad. sc. *190*: 1255–1258.
BLEIER, H. 1928. Genetik und Cytologie teilweise und ganz steriler Getreidebastarde. Bibliogr. genet., Gravenh. *4*: 321–400.
CHAPMAN, V., and R. RILEY. 1955. Disomic addition of rye chromosome II to wheat. Nature, Lond. *175*: 1091–92.
COFFEY, L. C. 1956. Interspecific and intergeneric hybrids of *Triticum* and related genera. Iowa State Coll. J. Sc. *30*: 342–343.
DUSTIN, A. P. 1934. Contribution à l'étude de l'action des poisons caryoclasiques sur les tumeurs animales: Action de la colchicine sur le sarcome greffé, type Crocker, de la souris. Bull. Acad. roy. méd. Belgique *14*: 487–502.
EIGSTI, O. J., and PIERRE DUSTIN, Jr. 1955. Colchicine in Agriculture, Medicine, Biology and Chemistry. Ames, Iowa: Iowa State Coll. Press, Pp. 470.
ELLIOTT, F. C. 1957. X-ray induced translocation of *Agropyron* stem rust resistance to common wheat. J. Hered. *48*: 77–81.
FLORELL, V. H. 1931. A cytologic study of wheat × rye hybrids and back crosses. J. Agr. Res. *42*: 341–362.
FUKASAWA, H. 1949. On the progenies of new hexaploid wheat plants. Jap. J. Genet. *24*: 6–7.
GOULDEN, C. H. 1929. Breeding rust resistant varieties of wheat: Fundamental aspects of the problem. Sc. Agr. *10*: 258–267.
——— 1956. Wheat breeding and production in Canada. World Crops *8*: 423–427.
HARRINGTON, J. B., and W. K. SMITH. 1929. The inheritance of reaction to black stem rust of wheat in a *dicoccum* × *vulgare* cross. Canad. J. Res. *1*: 163–188.
HAYES, H. K., J. H. PARKER, and C. KURTZWEIL. 1920. Genetics of rust resistance in crosses of varieties of *Triticum vulgare* with varieties of *T. durum* and *T. dicoccum*. J. Agr. Res. *19*: 523–542.
HEYNE, E. G., and R. W. LIVERS. 1953. Monosomic analysis of leaf rust reaction, awnedness, winter injury and seed color in Pawnee wheat. Agron. J. *45*: 54–58.
HYDE, BEALE B. 1953. Addition of individual *Haynaldia villosa* chromosomes to hexaploid wheat. Am. J. Bot. *40*: 174–182.
JENKINS, B. C. 1951. Substitutions of rye chromosomes in winter wheat. Proc. 6th Mtg. Ass. Com. Pl. Breed. Nat. Res. Counc. Canada. Y-2.
——— 1957. Radiation induced stem rust resistant mutants in durum wheat. Robigo Castelar *3*: 16–17.
JESENKO, F. 1913. Ueber Getreide-Speziesbastarde (Weizen-Roggen). Zschr. indukt. Abstamm. *10*: 311–326.
KAGAWA, F. 1939. The effect of abnormal temperature on the course of pollen formation in a genus hybrid *Triticum compactum* × *Secale cereale*. Jap. J. Bot. *10*: 55–68.
KIHARA, H. 1924. Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die Sterilität in den Bastarden. Mem. Coll. Sc. Kyoto Imp. Univ. *1* (B): 1–200.
——— 1944. Die Entdeckung des DD-Analysators beim Weizen. Agr. Hort. Japan *19*: 889–890.
KIHARA, H., and Y. KATAYAMA. 1931. Genomanalyse bei Triticum und Aegilops. III. Zur Entstehungsweise eines neuen konstanten oktoploiden *Aegilotricum*. Cytologia *2*: 234–255.
KNOTT, D. R. Personal communication.
KONZAK, C. F.; N. E. BORLAUG; A. ACOSTA; and J. GIBLER. 1956. Stripe rust resistant mutants obtained from irradiation of Gabo wheat. Phytopathology *46*: 525–526.
KOSTOFF, DONTCHO. 1937. Chromosome behaviour in *Triticum* hybrids and allied genera. Akad. Nauk. SSSR. Trudy Inst. Genet. *11*: 17–32.
——— 1941. Das Verhalten der Chromosomen in Weizenbastarden. IV. Die natürlichen Gattungsbastarde *Triticum timopheevi* × *Agropyron repens*. Züchter *13*: 269–272.
KUSPIRA, JOHN, and JOHN UNRAU. Unpublished data.
KUSPIRA, JOHN, and JOHN UNRAU. 1957. Genetic analyses of certain characters in common wheat using whole chromosome substitution lines. Canad. J. Pl. Sc. *37*: 300–326.
LEBEDEFF, V. N. 1934. Neue Fälle der Formierung von Amphidiploiden in Weizen-Roggen Bastarden. Zschr. Züchtung Reihe A. Pflanzenzücht. *19*: 509–525.

LEDINGHAM, G. F., and W. P. THOMPSON. 1938. The cytogenetics of non-amphidiploid derivatives of wheat-rye hybrids. Cytologia *8*: 377–397.
LEIGHTY, C. E., and W. J. SANDO. 1928. Natural and artificial hybrids of a Chinese wheat and rye. J. Hered. *19*: 23–27.
LEWITZKY, G. A., and G. K. BENETZKAJA. 1932. Cytology of the wheat-rye amphidiploids. Bull. Appl. Bot., Genet., and Pl. Breed. Ser. II. *27*: 241–264.
MARSHALL, H. G., and J. W. SCHMIDT. 1954. A study of the meiotic stability of certain *Agrotricum* hybrids. Agron. J. *46*: 383–388.
MATSUMURA, SEIJI. 1951. Genetics of some cereals. Annual Rep. Nat. Inst. Genet. Japan *1*: 22–27.
MCFADDEN, E. S. 1930. A successful transfer of emmer characters to vulgare wheat. J. Am. Soc. Agron. *22*: 1020–1034.
MCFADDEN, E. S., and E. R. SEARS. 1944. The artificial synthesis of *Triticum spelta*. Rec. Genet. Soc. Amer. *13*: 26–27.
——— 1947. The genome approach in radical wheat breeding. J. Am. Soc. Agron. *39*: 1011–1026.
MCGINNIS, R. C. Personal communication.
MEISTER, G. K. 1921. Natural hybridization of wheat and rye in Russia. Russ. J. Hered. *12*: 467–470. (Original not seen.)
MÜNTZING, ARNE. 1935. Triple hybrids between rye and two wheat species. Hereditas *20*: 137–160.
——— 1936. Ueber die Entstehungsweise 56 chromosomiger Weizen-Roggen-Bastarde. Züchter *8*: 188–191.
——— 1950. Die ökonomische Bedeutung und theoretische Grundlage der Schwedischen Pflanzenzüchtung. Züchter *20*: 1–8.
NEBEL, B. R., and M. L. RUTTLE. 1938. The cytological and genetical significance of colchicine. J. Hered. *29*: 3–9.
NYQUIST, W. E. 1957. Monosomic analysis of stem rust resistance of a common wheat strain derived from *Triticum timopheevi*. Agron. J. *49*: 222–223.
OEHLER, ERNST. 1931. Untersuchungen über Ansatzverhältnisse, Morphologie und Fertilität bei Weizen-Roggenbastarden. Zschr. Züchtung Reihe A. Pflanzenzücht. *16*: 357–393.
——— 1936. Untersuchungen an einem neuen konstant-intermiären additiven *Aegilops*-Weizenbastard (*Aegilotricum triuncialis-durum*). Züchter *8*: 29–33.
OHLENDORF, A. 1955. Weitere cytologische Untersuchungen an Weizen-Quecken-Bastarden. Züchter *25*: 331–351.
OHLENDORF, A. WIENHUES. 1957. Ergebnisse der Bastardierung von *Triticum timopheevi* mit Kultursorten des Weizens unter besonderer Berücksichtigung der Krankheitresistenz. Züchter *27*: 132–140.
OKAMOTO, M. 1957. Asynaptic effect of chromosome V. Wheat Inf. Serv. *5*: 6.
O'MARA, J. G. 1953. The cytogenetics of *Triticale*. Bot. Rev. *19*: 587–605.
PETO, F. H. 1936. Hybridization of *Triticum* and *Agropyron*. II. Cytology of the male parents and F_1 generation. Canad. J. Res. (Ser. C.) *14*: 203–214.
PISSAREV, V. E. 1957. Selektion für höheren Proteingehalt bei Sommerweizen. Züchter *27*: 371–375.
PISSAREV, V. E., and N. M. VINOGRADOVA. 1944. Hybrids between wheat and *Elymus*. Akad. Nauk. Dok. SSSR. *45*: 129–132.
RAGULIN, A. A. 1947. A hybrid between *Triticum durum* and *Elymus arenarius*. Akad. Nauk. Dok. SSSR. *55*: 251–253.
RILEY, RALPH, and VICTOR CHAPMAN. 1957. The comparison of wheat-rye and wheat-*aegilops* amphidiploids. J. Agr. Sc. *49*: 246–250.
RILEY, R., and V. CHAPMAN. Personal communication.
SARKAR, P., and G. L. STEBBINS. 1956. Morphological evidence concerning the origin of the B genome in wheat. Am. J. Bot. *43*: 297–304.
SAVCHENKO-BELSKY, A. 1948. New grain varieties, Tsitsin's perennial wheat. Soviet News *1981*: 4. (Original not seen.)
SAX, KARL. 1923. The relation between chromosome number, morphological characters and rust resistance in segregates of partially sterile wheat hybrids. Genetics *8*: 301–321.
SAX, KARL, and E. F. GAINES. 1924. A genetic and cytological study of certain hybrids of wheat species. J. Agr. Res. *28*: 1017–1032.
SEARS, E. R. 1939. Cytogenetic studies with polyploid species of wheat. I. Chromosomal aberrations in the progeny of a haploid of *Triticum vulgare*. Genetics *24*: 509–523.

——— 1944. Cytogenetic studies with polyploid species of wheat. II. Additional chromosomal aberrations in *Triticum vulgare*. Genetics *29*: 232–246.
——— 1948. The cytology and genetics of the wheats and their relatives. Advance. Genet. *2*: 239–270.
——— 1953a. Addition of the genome of *Haynaldia villosa* to *Triticum aestivum*. Am. J. Bot. *40*: 168–174.
——— 1953b. Nullisomic analysis in common wheat. Am. Natur. *87*: 245–252.
——— 1956a. The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Sympos. Biol. *9*: 1–22.
——— 1956b. The B genome of Triticum. Wheat Inf. Serv. *4*: 8–10.
Sears, E. R., W. Q. Loegering, and H. A. Rodenhiser. 1957. Identification of chromosomes carrying genes for stem rust resistance in four varieties of wheat. Agron. J. *49*: 208–212.
Sears, E. R., and H. A. Rodenhiser. 1948. Nullisomic analysis of stem rust resistance in *Triticum vulgare* var. Timstein. Genetics *33*: 123–124. (Abstract)
Smith, D. C. 1942. Intergeneric hybridization of cereals and other grasses. J. Agr. Res. *64*: 33–47.
Sorokina, O. N. 1937. A fertile and constant 42-chromosome hybrid *Aegilops ventricosa* Tausch. × *Triticum durum* Desf. Tr. priklad. bot. genet. selektsii. *7*: 5–12.
Stebbins, G. L., Jr., and Ting Fung Pun. 1953. Artificial and natural hybrids in the Gramineae tribe Hordeae. VI. Chromosome pairing in *Secale cereale* × *Agropyron intermedium* and the problem of genome homologies in the Triticinae. Genetics *38*: 600–608.
Thompson, W. P. 1925. The correlation of characters in hybrids of *Triticum durum* and *Triticum vulgare*. Genetics *10*: 285–304.
——— 1926. Chromosome behaviour in a cross between wheat and rye. Genetics *11*: 317–332.
——— 1931. Chromosome homologies in wheat, rye and *aegilops*. Canad. J. Res. *4*: 624–634.
Thompson, W. P., and L. Hollingshead. 1927. Preponderance of dicoccum-like characters and chromosome numbers in hybrids between *Triticum dicoccum* and *Triticum vulgare*. J. Genet. Cambr. *17*: 283–307.
Thompson, W. P., T. J. Arnason, and R. M. Love. 1935. Some factors in the different chromosome sets of common wheat. Canad. J. Res. *12*: 335–345.
Tschermak, E. 1923. Bastardierung zwischen relativ fremden Formen. Handb. landw. Pflanzenzücht. *4*: 198–199.
——— 1931. Ueber die praktische Verwendbarkeit der Weizen-Roggenbastarde. Deut. landw. Presse *58*: 29–30.
Tschermak, E. and H. Bleier. 1926. Ueber fruchtbare *Aegilops*-Weizenbastarde. Ber. Deut. bot. Ges. *44*: 110–132.
Tsitsin, N. 1946. Perennial wheats. Discovery, Lond. *7*: 180.
Unrau, John. 1950. The use of monosomes and nullisomes in cytogenetic studies of common wheat. Sc. Agr. *30*: 66–89.
Unrau, John, Clayton Person, and John Kuspira. 1956. Chromosome substitution in hexaploid wheat. Canad. J. Bot. *34*: 629–640.
Von Berg, K. Heinz, and Ernst Oehler. 1938. Untersuchungen über die Cytogenetik amphidiploider Weizen-Roggen-Bastarde. Züchter *10*: 226–238.
Wakar, B. A. 1935. Cytologische Untersuchungen der ersten Generation der Weizen-Queckengrasbastarde. Züchter *7*: 199–206.
White, W. J. 1940. Intergeneric crosses between *Triticum* and *Agropyron*. Sc. Agr. *21*: 198–232.
Wiggin, H. Carvel. 1955. Monosomic analysis of stem rust reaction and awn expression in Kentana 52 wheat. J. Hered. *46*: 239–242.
Zhebrak, A. 1944. Synthesis of new species of wheats. Nature, Lond. *153*: 549–551.

FERTILITY AND MORPHOLOGICAL VARIATION IN THE SUBSTITUTION AND RESTORATION BACKCROSSES OF THE HYBRIDS, *TRITICUM VULGARE* × *AEGILOPS CAUDATA*[1]

H. Kihara

AN EARLIER REPORT (Kihara, 1951) described wheat plants which had the hexaploid genome complement (VV) of *Triticum vulgare* (*T. aestivum*) in the plasma of *Aegilops caudata*. They had been started from two successive backcrosses of the hybrid *Aegilops caudata* × *T. vulgare* to *T. vulgare* as father. Then, since a third backcross was not available, the offspring of a second backcross plant, obtained from open pollination, had to be used. From these experiments *vulgare*-plants with *caudata*-plasma have been obtained. They closely resembled *T. vulgare*, except for their partial male sterility and black ears. This latter character must have been inherited from *Ae. caudata*, since *T. vulgare* used in the experiments had yellow ears. The genome complement of the *vulgare* plants with black ears has been designated by V^bV^b.

The present report deals with a new series of substitution as well as restoration backcrosses, which started in 1949 and was supplemented by series in succeeding years (Kihara, 1954). Reciprocal hybrids between *T. vulgare* and *Ae. caudata* were used. The main purpose of the experiments was to observe the changes in respect to morphology and fertility taking place as restoration and substitution were carried out.

The backcrosses were carried out according to the following plan:

Successive substitution backcrosses (SB_{1-9})

Ae. caudata	× *T. vulgare*	F_1	(1949)
F_1	× *T. vulgare*	SB_1	(1950)
SB_1	× *T. vulgare*	SB_2	(1951)
SB_2	× *T. vulgare*	SB_3	(1952)
..	.. .. ..	..	..
..	.. .. ..	..	..
..	.. .. ..	..	..
SB_8	× *T. vulgare*	SB_9	(1958)

Successive restoration backcrosses (RB_{1-9})

T. vulgare	× *Ae. caudata*	F_1	(1949)
F_1	× *T. vulgare*	RB_1	(1950)
RB_1	× *T. vulgare*	RB_2	(1951)
RB_2	× *T. vulgare*	RB_3	(1952)
..	.. .. ..	..	..
..	.. .. ..	..	..
..	.. .. ..	..	..
RB_8	× *T. vulgare*	RB_9	(1958)

From 1951 on F_1-hybrids between several strains of 6*x*-wheat and one strain of *Ae. caudata* were produced every year. They were backcrossed successively to the *vulgare*-parent as father, the first of them for seven backcross generations (RB_7).

[1]Contribution from the National Institute of Genetics, Misima, Japan, No. 253.

These repeated experiments, which sometimes led to new findings, were very useful in providing more accurate segregation ratios.

From time to time backcross plants were selfed or outcrossed to investigate their genotypic make-up.

The materials employed in the backcrosses are:

(1) Hexaploid wheat ($2n = 42$)
  (i) *T. vulgare* var. *erythrospermum*
  (ii) P 174 (a pure strain originated from SB_2, whose ears are black like *caudata*'s; the plasma is *caudata*; fertility is 60 per cent)
  (iii) P 168 (a pure strain obtained from the cross between *T. vulgare* var. *erythrospermum* and P 174; the plasma is *vulgare*)
(2) *Aegilops caudata* ($2n = 14$)
(3) F_1
  (i) *T. vulgare* var. *erythrospermum* × *Ae. caudata* (rec.)
  (ii) P 174 × *Ae. caudata*
  (iii) P 168 × *Ae. caudata* (rec.)

Vulgare-plasma was designated by β, *vulgare*-genome complement (ABD) by V, *caudata*-plasma by α^c and *caudata*-genome by C. Accordingly, the plasma-genome symbols for the *vulgare*-genome complement in its own and in *caudata*-plasma are βVV and α^cVV, respectively. Those for P 174 and P 168 are $\alpha^c V^b V^b$ and $\beta V^b V^b$, respectively. Selfed lines are indicated by s added as superscript to the symbols of backcross generations: for example, the selfed offspring of RB_2 is $(RB_2)^{S1}$ and the next generation obtained again by selfing is $(RB_2)^{S2}$.

Chromosome Behavior and Chromosome Numbers in F_1 and Successive Backcross Generations

As far as the chromosome behavior in F_1 and the procedure of restoration or substitution in the subsequent generations are concerned, all hybrid combinations were quite similar. Since, as will be shown later, P 174 and P 168 were supposed to have one chromosome of *Ae. caudata* (C-Sat-2), the number of bivalents in the two F_1-combinations, P 174 × *Ae. caudata* and P 168 × *Ae. caudata*, should have exceeded by one that of the F_1-hybrid *T. vulgare* × *Ae. caudata*. The findings did not justify this assumption. Our descriptions are chiefly devoted to the reciprocal crosses, *T. vulgare* × *Ae. caudata*, whose successive backcrosses attained the ninth generation in 1958.

The F_1-hybrids had 0 to 6 bivalents in pollen-mother cells (PMC) at first metaphase (MI). The chromosome behavior in the reciprocal hybrids was quite similar (Table I, Fig. 1). Often restitution nuclei were observed which were expected to give rise to functional unreduced gametes. Accordingly, the first backcross offspring (SB_1 and RB_1) often had 49 chromosomes. However, many plants occurred which

TABLE I

Range of Bivalent Number in Reciprocal F_1-Hybrids between *Ae. caudata* and *T. vulgare* (1949)

Cult. No.	♀ ♂	Range of variation in bivalent number	Complexes	Remarks
29	*Ae.caudata* × *T.vulgare*	0–4 (or more) (mode 2)	$0–1_{III}$, $0–1_{IV}$	Often restitution nuclei
30	*T.vulgare* × *Ae.caudata*	0–5 (mode 3)	$0–1_{III}$	Often restitution nuclei

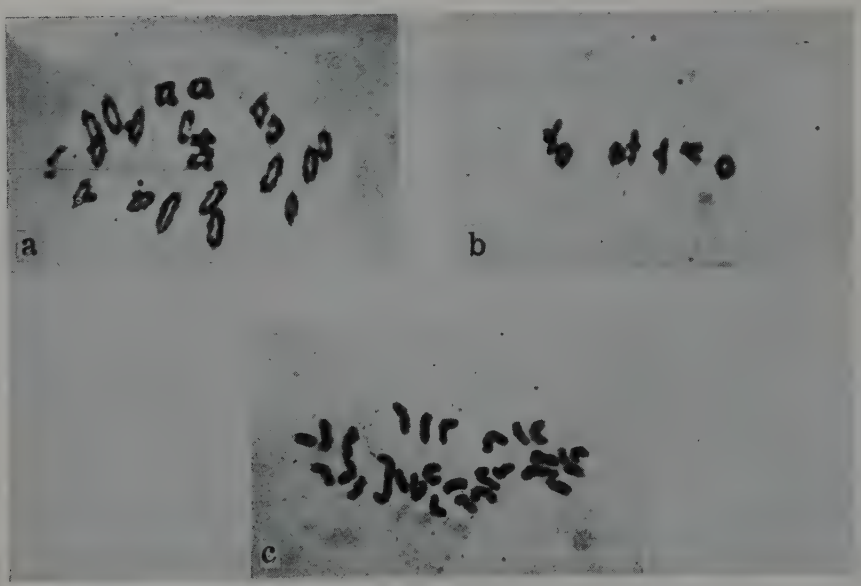


FIGURE 1. Chromosomes (PMC) at I metaphase in (*a*) *T. vulgare*; (*b*) *Ae. caudata*; (*c*) F_1 = *T. vulgare* × *Ae. caudata.*

possessed various other chromosome numbers (36 to 52). Table II shows the variation in the diploid chromosome numbers in SB_1 and RB_1 which were repeatedly obtained during the past six years.

In Table III, chromosome-pairing in PMC of 16 B_1-plants is given. From the chromosome configuration of the respective B_2-plants, the number of *caudata* chromosomes in F_1-gametes was estimated. It is highly probable that the number of *caudata*-chromosomes is very often seven and that the whole C-genome was present in the F_1-gametes. In agreement with this cytological finding, three dominant

TABLE II

FREQUENCY OF B_1-INDIVIDUALS HAVING 36–52 CHROMOSOMES IN THE FIRST BACKCROSS: F_1 × *T. vulgare* IN THE YEARS 1950–7

		Chromosome numbers (2*n*)																	
	B_1	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	Total
1950	SB_1		1	1	1	1			1			1		1	2	1			10
	RB_1									1		1	1		3				6
1952	SB_1(168)*					1		1				1	2	2	1				8
	SB_1(174)*														1				1
	RB_1	1					1	1			1	1	1	1	1				8
	RB_1(168)*					1			2			1	1		3	1			9
1954	RB_1	1													1				2
	RB_1(168)*			1															1
1955	RB_1														1				1
1956	RB_1												1	1	5	1		1	9
1957	RB_1											1	1						2
	TOTAL	2	1	2	1	3	1	2	3	1	1	6	7	5	18	3	0	1	57

*168 = $\beta V^b V^b$ and 174 = $\alpha^c V^b V^b$.

TABLE III

CHROMOSOME PAIRING IN RB_1- AND SB_1-PLANTS AND ESTIMATED NUMBERS OF *caudata*-CHROMOSOMES IN F_1-GAMETES

No. of plants		$2n$	Configuration (PMC)	Number of *caudata*-chromosomes calculated from the configurations
RB_1	56–1	47	$19_{II}+9_I$	7
	–2	49	$21_{II}+7_I$	7
	–3	46	$20_{II}+6_I$	5
	–4	49	$21_{II}+7_I$	7
RB_1	57–1	49	$21_{II}+7_I$	7
	–2	44	$16_{II}+12_I$ ($17_{II}+10_I$)	6–7
SB_1	58–1	37	$12_{II}+13_I$	4
	–2	48	$20_{II}+8_I$	7
	–3	40	$14_{II}+12_I$	5
	–4	39	$13_{II}+13_I$	5
	–5	46	$19_{II}+8_I$	6
	–6	38	$10_{II}+18_I$	7
SB_1	59–1	43	$13_{II}+17_I$	7
	–2	49	$21_{II}+7_I$	7
	–4	49	$21_{II}+7_I$	7
	–5	50	$21_{II}+8_I$	7 or more

caudata-characters were very frequently found among the B_1-plants. The percentages of plants with three such characters are shown in Table IV.

For further investigations 49-chromosome B_1-plants were selected, in order to start with a material as uniform as possible. The chromosome conjugation in these B_1-plants was quite similar. The most frequently found chromosome configuration was $21_{II} + 7_I$. The pairing was to some extent subject to variation (Table V). The number of bivalents varied from 19 to 21 and the trivalents from 0–3 (Fig. 2).

TABLE IV

PERCENTAGE OF B_1-PLANTS WITH THREE DOMINANT *caudata*-CHARACTERS (1950)

Organ	Characters		Percentage
	caudata	*vulgare*	
Ears	Black	Yellow	
	114	10	91.9
Whole plants	Non-waxy	Waxy	
	125	16	88.7
Stems	Purple	Yellow	
	87	23	79.1

TABLE V

CHROMOSOME CONJUGATION IN THREE B_1-PLANTS ($2n = 49$)

	Cult. no.	$2n$	Chromosome conjugation	Modifications
RB_1	57–1	49	$21_{II}+7_I$	$(19–21)_{II}$
SB_1	59–2	49	$21_{II}+7_I$	$(0–3)_{III}$
SB_1	59–4	49	$21_{II}+7_I$	$(0–1)_{IV}$

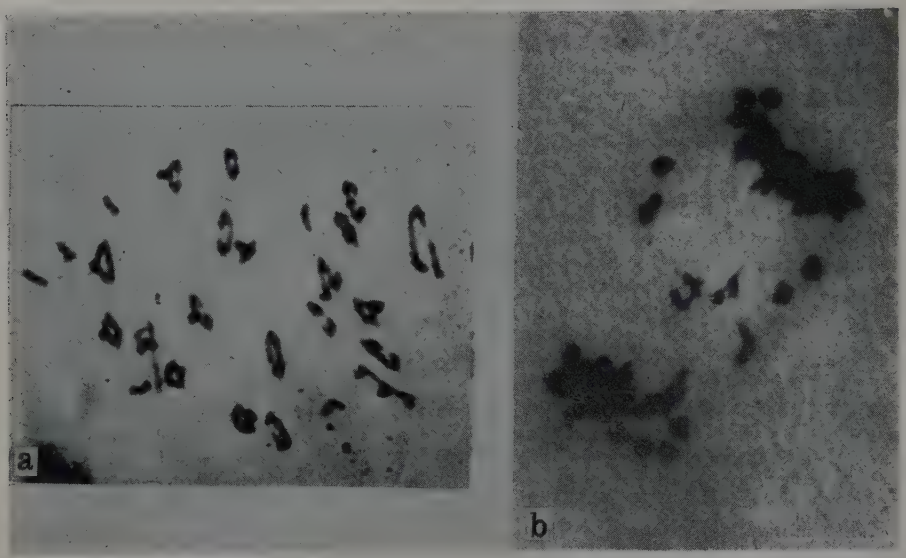


FIGURE 2. Chromosomes (PMC) of RB_1 57-1; (*a*) I metaphase with $3_{III} + 17_{II} + 6_I$; (*b*) I anaphase.

One tetravalent could seldom be found. The meiotic chromosomes showed a tendency to stickiness, especially in SB_1, and accordingly, many chromosome fragments were found in the next generation, especially in the root-tip cells. Plants with fragments were not used in backcrosses; therefore they will be left out of consideration in this report.

In one 49-chromosome $(RB_1)^{S1}$-plant, the variation in the number of bivalents was very wide ranging from 17 to 23. Mode was at 22. Plants in which the number of bivalents exceeded 21 have been observed in B_2 also. For instance, one RB_2-plant (cult. no. 14-5) had 19 to 22 bivalents. The most frequent were $22_{II} + 2_I$. The nature of such supernumerary pairing will be discussed later (p. 168).

From the backcrosses, $SB_1 \times T.$ *vulgare* and $RB_1 \times T.$ *vulgare*, offspring with 42 to 49 chromosomes were expected. This expectation was fulfilled, as shown in Table VI. Plants with 49 chromosomes were missing in 1951, but two such plants appeared in the progeny of two selfed B_1-plants. The absence of 49-chromosome plants in 1951 can be considered as due to chance. In 1956 one 49-chromosome RB_2 plant was found. Five B_2-plants with 41 and one with 40 chromosomes were found. Their rare appearance can be predicted from the number of bivalents in 49-chromosome B_1-plants.

To obtain B_3, many B_2-plants were used, but the description in this report will be restricted to a few representative ones.

In the substitution series one of the SB_2-plants used in producing SB_3 had $21_{II} + 3_I$ ($2n = 45$, cult. no. 24-7) and another one 21_{II} ($2n = 42$, cult. no. 27-7) (Figs. 3a and b). The former plant showed a varying number of bivalents, 21 occurring most frequently; plates with $20_{II} + 5_I$ or $1_{III} + 19_{II} + 4_I$ were also observed. Therefore, it was expected that SB_3 obtained from this plant would have varying chromosome numbers. The examined offspring had 41 to 43 chromosomes with the mode at 42 (mode for pairing at 21_{II}). The latter (cult. no. 27-7) showed variation in chromosome-pairing also. Up to four univalents were observed, most

TABLE VI

FREQUENCY OF B_2-INDIVIDUALS WITH 40–49 $2n$ CHROMOSOMES

	Restoration or substitution backcrosses	Somatic chromosome numbers of B_2										Total
		40	41	42	43	44	45	46	47	48	49	
1951	RB_2		1		6	11	4	1	2	1		26
	SB_2			1	13	7	11	9	3			44
1953	RB_2*			(3)		(1)						(4)
	RB_2			2	3	1		1				7
1954	RB_2		2	1	3	2	1	2				11
	RB_2 (168)		1		1	1	1					4
	RB_2 (174)			1		1	3	1				6
1955	RB_2				4	2	2					8
1956	RB_2		1	4	4	15	11	4	2	1	1	43
1957	RB_2	1		1	6	5	2	4	1			20
	TOTAL	1	5	10	40	45	35	22	8	2	1	169

*This RB_2 was obtained from the cross, B_1 ($2n = 48$) × *T. vulgare*. Number of B_2-plants from this cross was not added to the total. For all other backcrosses, B_1-plants having $2n = 49$ were used. RB_2 (168) and SB_2 (174) show that in the F_1-hybrid and in the succeeding backcross generations, strains "P 168" ($\beta V^b V^b$) and "P 174" ($\alpha^c V^b V^b$) were used respectively.

frequently $21_{II} + 0_I$. Accordingly, we have obtained from this plant SB_3-offspring with 41 to 43 chromosomes (Table VII).

In the restoration series one of the plants used in the backcrosses had at MI $1_{III} + 20_{II}$ or $21_{II} + 1_I$ ($2n = 43$; cult. no. 11-6), and another one had $21_{II} + 1_I$ (Figs. 3c and d). The RB_3-offspring obtained from these plants had mostly 21 bivalents at MI. In Figure 4 metaphase plates of two SB_3- and two RB_3-plants are shown.

In the production of the fourth backcross generation only those B_3-plants which were found to form 21_{II} at MI were used. Accordingly, both backcross generations,

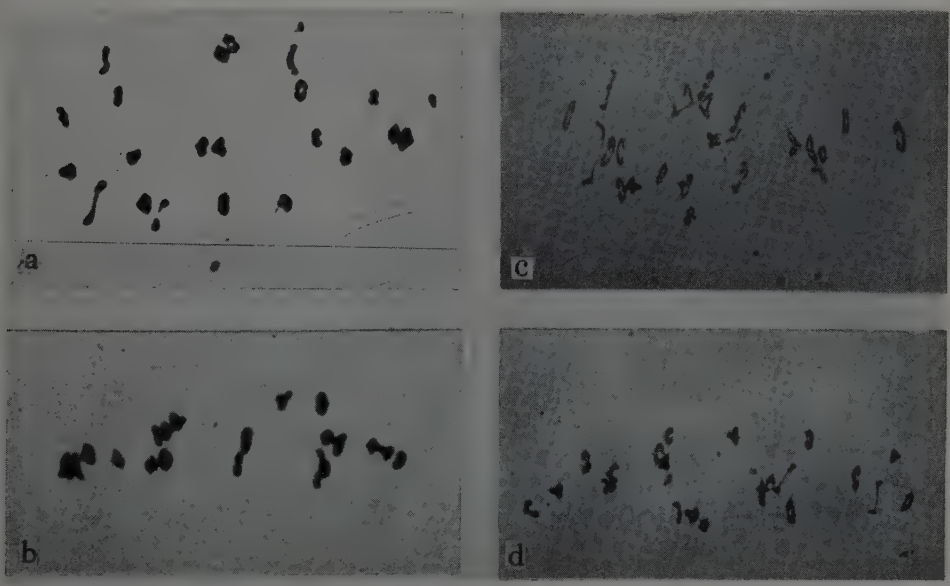


FIGURE 3. Chromosomes (PMC) at I metaphase: (*a*) SB_2 24-7, $45 = 21_{II} + 3_I$; (*b*) SB_2 27-7, $42 = 21_{II}$; (*c*) RB_2 11-6, $43 = 1_{III} + 20_{II}$; (*d*) RB_2 14-6, $43 = 21_{II} + 1_I$.

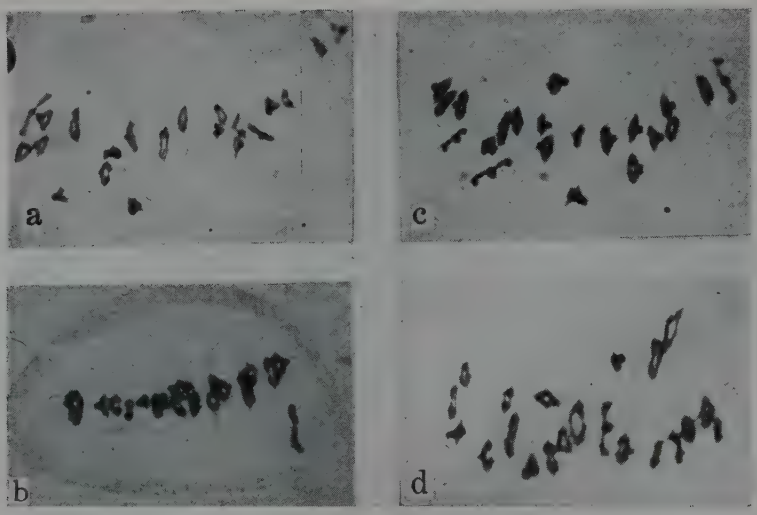


FIGURE 4. Chromosomes (PMC) at I metaphase, all showing 21_{II}: (*a*) SB_3 45-5; (*b*) SB_3 51-1 (one heteromorphic pair outside the plate); (*c*) RB_3 37-2; (*d*) RB_3 41-3.

SB_4 and RB_4, consisted of 42-chromosome plants with 21_{II} at MI. With regard to chromosome behavior during meiosis, there was no difference between the SB_4- and the RB_4-offspring (Fig. 5).

In the following backcross generations ($B_5 \sim B_9$), 21 bivalents were almost always seen. Very seldom were two univalents observed at MI, as they are found to occur also at first metaphase of *T. vulgare*.

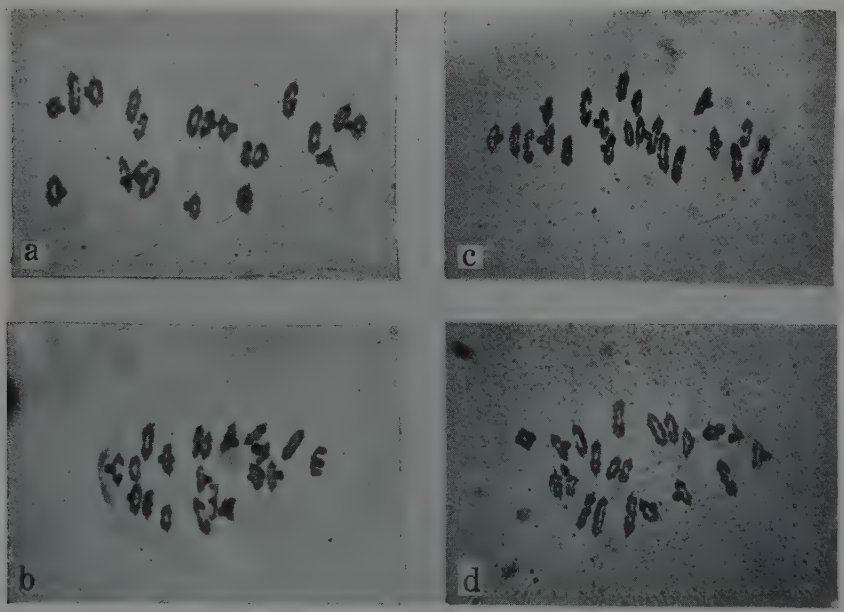


FIGURE 5. Chromosomes (PMC) at I metaphase, showing 21_{II}: (*a*) SB_4 86-8; (*b*) SB_4 93-2; (*c*) RB_4 74-5; (*d*) RB_4 81-5.

Karyotypes

T. vulgare is characterized by two sets of Sat-chromosomes (four Sats). The trabant of Sat-1 is larger and the middle portion longer than that of Sat-2. The remaining *vulgare*-chromosomes are V- or L-shaped.[2] *Ae. caudata* has in the haploid complement two Sat-chromosomes (C-Sat-1and C-Sat-2), two J- and three i-shaped chromosomes (Senjaninova-Korczagina, 1932; Kihara and Kondo, 1943). Both P 174 and P 168 have four Sats of *vulgare* and two smaller Sats. It was assumed that one *vulgare*-chromosome has been replaced by C-Sat-2 in the course of substitution backcrosses followed by self-pollination (Fig. 6a).

FIGURE 6. (*a*) Somatic chromosomes of P 174 (2 Sat-1 + 2 Sat-2 + 2 C-Sat-2); (*b*) I metaphase plate of *T. vulgare* × P 174 showing a heteromorphic pair including C-Sat-2 as one of the two partners.

F_1 hybrids between *T. vulgare* and *Ae. caudata* did not give perfect figures of *caudata*-chromosomes. However, the F_1 from the two crosses, P 174 × *caudata* and P 168 × *caudata*, gave excellent preparations (Fig. 7). Two *vulgare* Sats, one C-Sat-1 and two C-Sat-2, were identified quite easily. C-Sat-1 is larger than C-Sat-2. The trabant of the former is usually widely separated from the short arm. The remaining five *caudata*-chromosomes were J_1, J_2, i_1, i_2, and i_3. Three i-shaped chromosomes are easily distinguished from the others by their size. But it is difficult to identify them if only one or two are found in later generations. J-shaped chromosomes are composed of two arms (short and long). J_1 is rather L-shaped while J_2 is truly J-shaped. The whole chromosome length of J_2 exceeds that of J_1.

Among others, B_1-plants with 49 chromosomes were analyzed in 1950. In No. 57-1, which was an RB_1-plant used in further restoration backcrosses, we could recognize with some difficulties the whole basikaryotype of the *caudata*-genome (two C-Sats, two J-, and three i-shaped), besides 42 *vulgare*-chromosomes. No. 59–2, which was an SB_1-plant used for substitution crosses, was not analyzed. In one

[2]A third Sat-chromosome was observed by Câmara (1943). This one has a small trabant. It was impossible to identify this Sat in my preparations.

FIGURE 7. Somatic chromosomes of F_1 (P 174 × *Ae. caudata*).

RB_1-plant (No. 56-2), which also had 49 chromosomes, only three i were clearly observed. The remaining four *caudata*-chromosomes could not be identified.

In 1952 and 1953 the analysis of B_2 and B_3 was very successful owing to the assistance of Dr. Cua. Two examples representing SB_{2-3} and RB_{2-3} are represented in Table VII. It is interesting to note that the 43-chromosome RB_2-plant (No. 11-6)

TABLE VII

KARYOTYPES AND PAIRING OF CHROMOSOMES IN TWO STRAINS OF B_2 AND B_3 (1951–52)

RB_2	Karyotype	Pairing	RB_3	Karyotype	Pairing
11–6	$2n = 43$	$1_{III}+20_{II}$ ——× *T. v.*	37–2	$2n = 42$	
	2 Sat-1	$21_{II}+1_I$		2 Sat-1	
	2 Sat-2			2 Sat-2	21_{II}, $20_{II}+2_I$
	1 C-Sat-1	$20_{II}+3_f$		1 C-Sat-1	
			37–4	$2n = 42$	
				1 Sat-1	
				2 Sat-2	21_{II}
				1 C-Sat-1	

SB_2	Karyotype	Pairing	SB_3	Karyotype	Pairing
27–7	$(2n = 42)$	21_{II} ——× P 168	50–1		
	not analyzed	$20_{II}+2_I$		2 Sat-1	21_{II}, $1_{IV}+19_{II}$
				1 Sat-2	
		$19_{II}+4_I$		1 C-Sat-2	(one heteromorphic pair
			–2	4 Sats	with Sat-2 and C-Sat-2)
				1 C-Sat-2	
			–4	4 Sats	21_{II}, $20_{II}+2_I$
				1 C-Sat-2	
		— × *T. v.*	51–1		
				2 Sat-1	21_{II}, $20_{II}+2_I$
				1 Sat-2	
			–3	3 Sats	$21_{II}+1_f$
			–4	4 Sats	21_{II}, $20_{II}+2_I$

had 4 Sats and 1 C-Sat-1, and often showed one trivalent and 20 bivalents. This plant gave many RB_3-plants. Only two plants (Nos. 37-2 and 37-4) with different karyotypes are given in Table VII. Figure 8a shows the karyotype of No. 37-2 (4 Sats + 1 C-Sat-1). It may be assumed that the trivalent in the RB_2 (No. 11-6) consisted of 2 Sat-1 and 1 C-Sat-1. If there is free assortment of the three chromo-

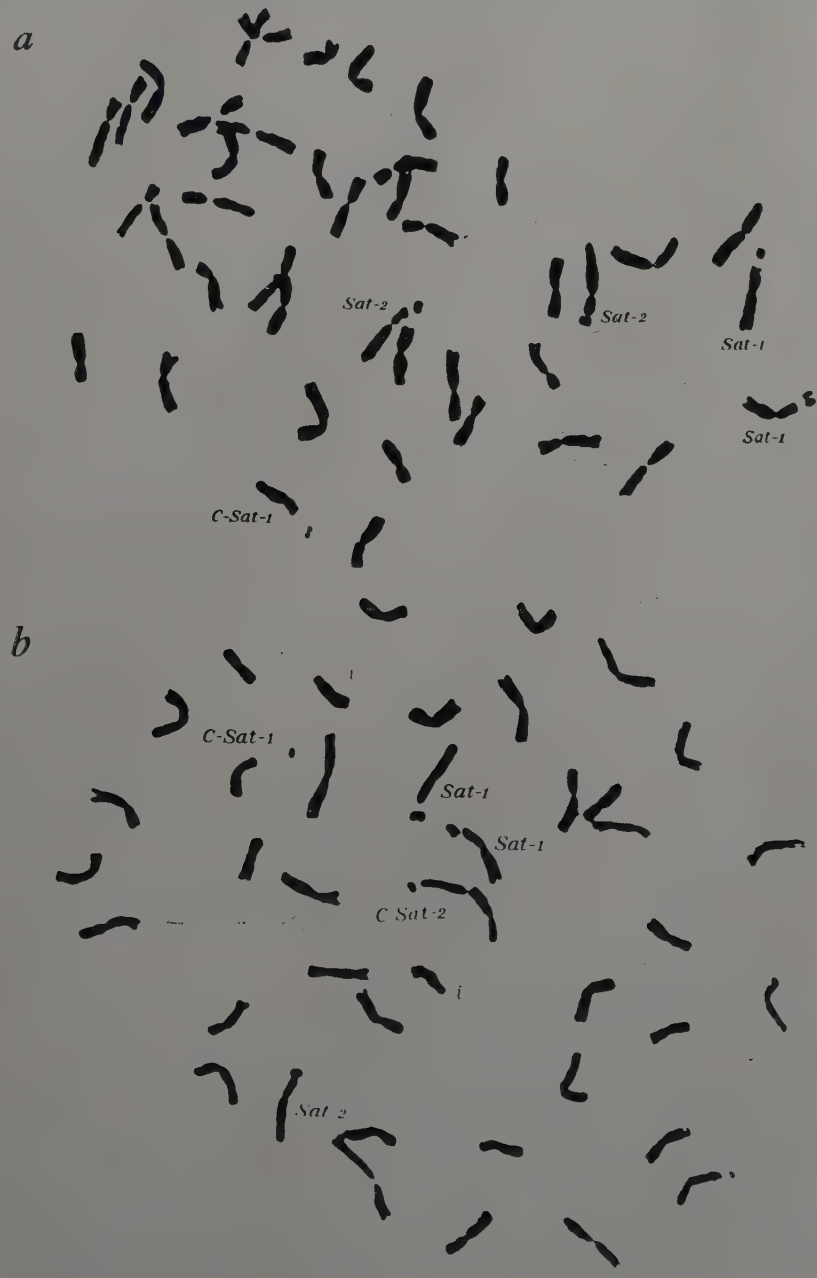


FIGURE 8. (*a*) Karyotype of RB_3 37-2 ('52), 2 Sat-1 + 2 Sat-2 + 1 C-Sat-1; (*b*) Karyotype of RB_1 22-3 ('52), 2 Sat-1 + 1 Sat-2 + 1 C-Sat-2 + 2 i.

somes during meiosis, we should expect 21-chromosome gametes with 1 C-Sat-1. The RB_3-plant No. 37-4 could have been obtained from such gametes. The pairing (21_{II}) in two RB_3-plants (Nos. 37-2 and 37-4) indicates that C-Sat-1 conjugates with one of non-Sat *vulgare*-chromosomes and Sat-1, respectively. The association in the former seems to be somewhat weak as plates with $20_{II} + 2_I$ are sometimes observed.

In SB_2-series, the karyotype of No. 27-7 was not analyzed. However, from the unstable pairing and the karyotypes of the progeny, its heterozygous condition is evident. This plant was crossed with P 168 and *T. vulgare*. From the first cross, 27-7 × P 168, two different karyotypes were obtained. The second cross (SB_3-progeny) also produced two karyotypes. The difference between these two crosses consists in the presence of 1 C-Sat-2 in the first cross which was contributed by P 168.

SB_2-plant No. 27-7 seems to have in its karyotype 2 Sat-1 + 1 Sat-2 (1 Sat-2 missing). SB_3-plants, 51-1 and most likely 51-3, have the same karyotype. As these plants have 21 bivalents at MI, the Sat-2 must conjugate with a *caudata*-chromosome other than the Sats. As could be expected, among 21 bivalents of 51-1, one heteromorphic pair is clearly seen (Fig. 4b).

Illegitimate chromosome conjugation is rather common in our materials. P 174 × *T. vulgare* and P. 168 × *T. vulgare* have 4 Sats and 1 C-Sat-2 in somatic cells, and 21_{II} including one heteromorphic pair are clearly observed at MI (Fig. 6b). This heteromorphic pair can be attributed to the conjugation of C-Sat-2 with one *vulgare*-chromosome. In 100 PMC of P 174 × *T. vulgare* only two plates had $20_{II} + 2_I$. In comparison, three plates with $20_{II} + 2_I$ were observed in 100 PMC of *T. vulgare*.

Figure 8b shows a somatic plate of an RB_1-plant (22-3) with 42 chromosomes. The karyotype of this plant is 2 Sat-1 + 1 Sat-2 + 1 C-Sat-1 + 1 C-Sat-2 + 2 i besides 35 other *vulgare*-chromosomes. The conjugation in PMC is $17_{II} + 8_I$, $18_{II} + 6_I$ and $19_{II} + 4_I$. Eight univalents of the configuration ($17_{II} + 8_I$) might have been derived from two i, two C-Sats and four *vulgare*-chromosomes. If so, from semi-homologous conjugation of two C-Sats and two *vulgare*-chromosomes the configuration $19_{II} + 4_I$ could be easily obtained. As could be expected from the karyotype, up to two trivalents were found. The two semi-homologous pairs found in this plant might have had a weakening effect upon the strength of the pairing.

Morphology

Eight characters were selected as markers of *caudata*-chromosomes: (1) fragility —the ear of *caudata* when ripe disarticulates at the base, above a few sterile spikelets, and breaks off as a whole; (2) black ears; (3) non-waxy:[3] ears, stems, and leaves of *Ae. caudata* have a non-waxy cuticle; one dominant gene is responsible for the inhibition of the development of wax; (4) purple stem; (5) winter habit; (6) late maturity; (7) short culm; (8) profuse tillering. In F_1 and B_1 characteristics (1) to (4) are dominant, while (5) to (8) are partially dominant. The latter are presumably controlled by two or more polymeric genes. With the completion of restoration or substitution all these characters should disappear. The presence of one of them in a 42-chromosome backcross-plant would indicate that the responsible gene or gene block, or even a whole *caudata*-chromosome carrying

[3]Waxless, a character found among some cultivated common wheats, is attributed to a recessive gene.

the gene in question, has been incorporated into the *vulgare*-genome. In the last case a whole *caudata*-chromosome would have replaced a *vulgare*-chromosome.

With respect to general morphology, the B_1-plants were not uniform, a fact which could be attributed to differences in the chromosome constitution. Only 49 chromosome B_1-plants were used in the production of further backcrosses.

In 1950 two SB_1- and three RB_1-plants with 49-chromosomes were under observation for the waxy character. Four of them were non-waxy, while one (an RB_1-plant) was waxy. Up to the present 15 B_1-plants with 49 chromosomes have been added. In Table VIII 18 49-chromosome as well as four supernumerary B_1-plants are listed. As these 22 B_1-plants must have had at least two *vulgare*-genome-complements and one *caudata*-genome, they should manifest dominant characters of *caudata*. However, among 18 49-chromosome B_1-plants, waxy individuals occurred three times and among supernumerary plants there was one yellow-eared plant.

The origin of 49-chromosome B_1 can be most probably attributed to the union of unreduced F_1 egg cells with male gametes of *T. vulgare*. Upon this assumption, the first backcross possessed the *vulgare*-genes in double and the *caudata*-genes in single doses. If they were always produced in this way, then we should expect a uniform B_1. But owing to crossing-over between the chromosomes of different genomes there could be some exceptions, where recessive characters would appear in B_1. In this case the genome constitution of the first backcrosses (SB_1 and RB_1) should be expressed by the formula V′VC′ in which V′ and C′ represent modifications of the original *vulgare*- and *caudata*-genomes, respectively.

TABLE VIII

MORPHOLOGY OF SB_1- AND RB_1-PLANTS WITH 49 (OR MORE) CHROMOSOMES

	No. of plants	2*n*	Waxy or non-waxy	Ear color	Stem color	Articulation	Anthers	Segregation in B_2 nw:w
1950	56–2(RB_1)	49	nw	( )	( )	( )	( )	
	56–4(RB_1)	49	nw	bl	( )	( )	( )	
	57–1(RB_1)	49	w	bl	( )	Not fragile (?)	( )	0 : 40
	59–2(SB_1)	49	nw	bl	( )	Fragile	( )	55 : 14(nw > w)
	59–4(SB_1)	49	nw	bl	( )	Fragile	( )	8 : 8
	59–5(SB_1)	50	nw	bl	( )	Fragile	( )	
1952	23–1(RB_1)	49	nw	bl	p	( )	−∼+++	10 : 13
	62–3(SB_1, 168)	49	nw	bl	p	( )	−∼+++	8 : 23(nw < w)
	65–2(RB_1, 168)	49	nw	bl	p	( )	−∼++	4 : 8
	66–2(RB_1, 168)	50	nw	bl	p	( )	−∼+++	
	68–2(RB_1, 168)	49	nw	bl	p	( )	+∼++	12 : 1(nw > w)
	71–1(RB_1, 168)	49	w	bl	p	( )	( )	
	74–2(SB_1, 174)	49	nw	bl	p	( )	—	4 : 9
1954	10–2(RB_1)	49	nw	bl	p	( )	−∼+++	2 : 7
1955	12–1(RB_1)	49	nw	bl	p	( )	( )	40 : 6(nw > w)
1956	10–1(RB_1)	52	nw	y	p	( )	−	
	10–2(RB_1)	50	nw	bl	p	( )	−∼+	
	10–6(RB_1)	49	nw	bl	p	( )	−∼++	2 : 7
	10–10(RB_1)	49	w	bl	p	( )	−∼++	
	10–12(RB_1)	49	nw	bl	p	( )	−∼++	12 : 2(nw > w)
	10–16(RB_1)	49	nw	bl	p	( )	−∼++	
	10–21(RB_1)	49	nw	bl	p	( )	−∼++	

nw = non-waxy leaves; w = waxy leaves; bl = black glume; y = yellow glume; p = purple stem; ( ) = not observed

− = anthers not dehiscent

+, ++, +++ = anthers partly to completely dehiscent

To investigate whether or not crossing-over between a *vulgare*-chromosome and a *caudata*-chromosome takes place, the non-waxy character was chosen. This character is clearly distinguishable before the flowering time, while black color and fragility of the ear are expressed after maturation.[4] Hence backcrossing or eventually selfing were conducted with the non-waxy character in view. There are two possible explanations for the occurrence of waxy plants in the 49-chromosome B_1: (1) crossing-over between the non-waxy *caudata*-chromosome and a corresponding *vulgare*-chromosome at prophase (for the production of unreduced gametes, see Fig. 9);

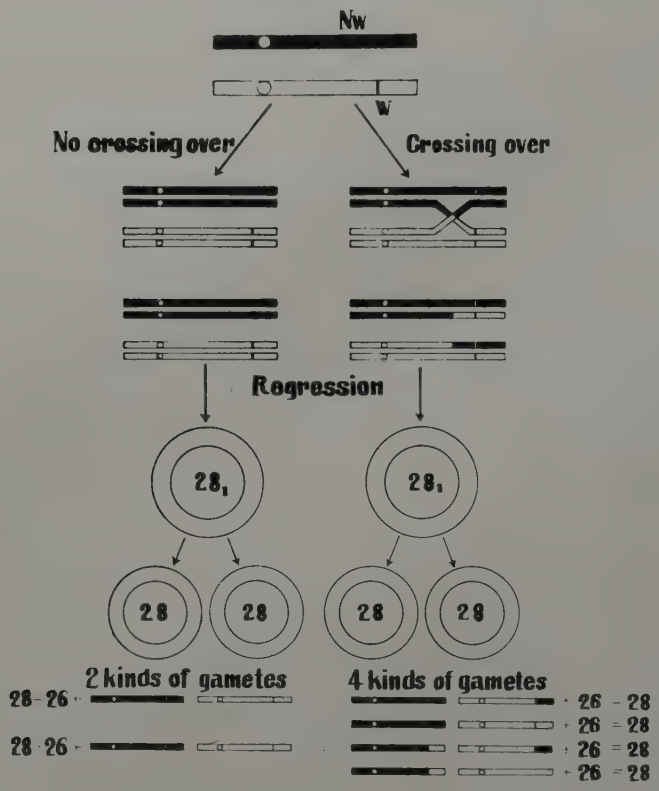


FIGURE 9. Diagram showing the formation of non-waxy and waxy gametes with 28 chromosomes by crossing-over between two semi-homologous chromosomes. The genotypes of four kinds of gametes are nwnw, nww, nww, and ww.

(2) formation of 28-chromosome gametes accompanied by non-disjunction of the non-waxy chromosomes (details shown in Fig. 10). Regarding the first possibility further explanation is needed. In 1958 I observed chromosomes of F_1 (P 174 $\times$ *caudata*) just after the last contraction stage. The number of paired chromosomes varied greatly. However, the maximum number of paired and end-to-end associated chromosomes was 14 as observed in one PMC (Fig. 11). I do not know yet whether all chromosomes are associated at prophase. With the advancing stages the

[4]One non-fragile 7*x*-individual recorded in 1951 will be kept out of consideration as the expression of this character is sometimes weakened owing to the sudden ripening of the plants under the summer conditions prevalent in Japan.

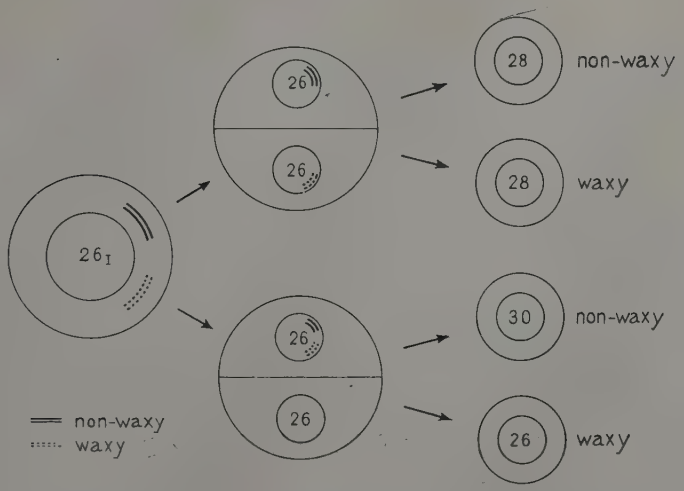


FIGURE 10. Formation of 28-chromosome gametes with waxy (or non-waxy) gene by non-disjunction of non-waxy *caudata*-chromosomes in the F_1-hybrid, *T. vulgare* × *Ae. caudata*.

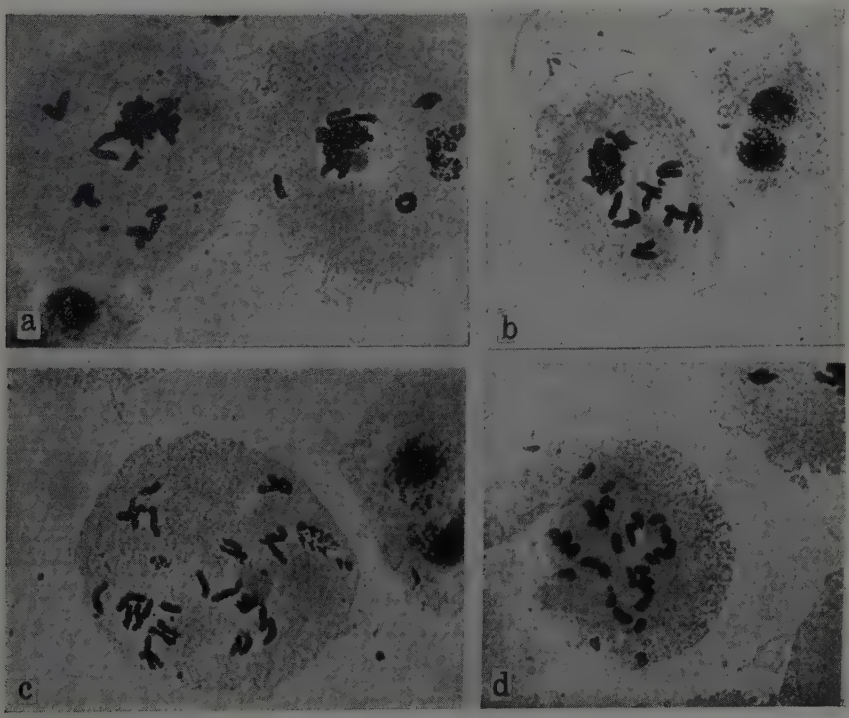
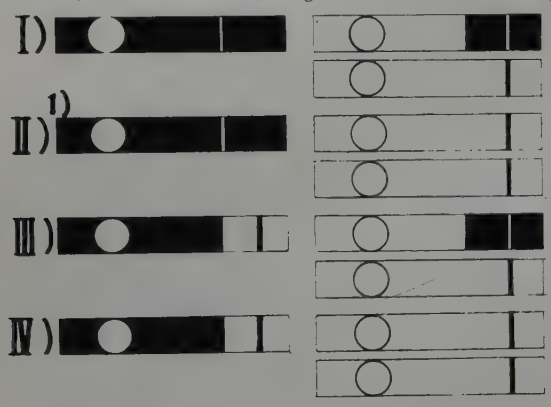


FIGURE 11. Chromosomes from last contraction stage (*a*,*b*,*c*) to early metaphase (*d*): (*a*,*b*) a part of chromosomes still in the contraction stage and the remaining scattered in the nuclear cavity mostly paired; (*c*) all chromosomes are paired or end-to-end loosely associated; one pair is attached to a nucleolus.

number of univalents increases and reaches its maximum at the metaphase. But still bivalents with terminal chiasma or chiasmata remain connected. All chromosomes are scattered after the nuclear membrane disappears. Soon they tend to assemble near the equatorial plate. In this hybrid the maximum number of bivalents and trivalents at MI was five and three respectively.

The first division of PMC is complicated and difficult to analyze. But usually the chromosomes separate into two or more daughter nuclei. The univalents go to the poles divided or undivided. Some of them do not reach the poles and form micronuclei. We often met with the phenomenon that all the univalents, including eventually bivalents, collapse into one big regression nucleus. Thus, the second division is morphologically equational. However, for two dyad chromosomes which arose from a crossover, the division may be partly reductional. Accordingly, four kinds of gametes will be produced (Fig. 12). In Figure 12, it is shown how four kinds of chromosome combinations in 49-chromosome B_1 will give different segregation ratios in B_2.



1) Without crossing over in F_1, B-gametes would produce only such B_2 ratio as given in category II.

FIGURE 12. Four kinds of chromosome combinations in 49-chromosome B_1-plants resulting in four different ratios of nw : w in B_2.

As to the second hypothesis, a full explanation is not needed. As F_1 gametes with more than 21 chromosomes were proved by the existence of 50- and 52-chromosome B_1-plants, non-disjunction of dyad chromosomes cannot be disregarded. However, the first hypothesis seems to be more probable, since it makes possible four different segregation ratios of non-waxy in B_2, while only two kinds of segregation ratios could be expected from the second assumption (nw > w and 100 per cent w).

Figure 13A shows the change in the segregation ratios throughout the successive substitution backcrosses. It lists also the actual figures from which the percentages were calculated. There is a considerable difference between two B_2-plants, which show two distinct segregation ratios (nw > w and nw < w). However, the ratio becomes 1:1 in a B_3, which was obtained from a B_2-plant with 21_{II}.

Figure 13B(d) shows the same relation as (nw < w) Figure 13A(b). But here it will be noted that one RB_3-strain obtained from an RB_2-plant with $21_{II} + 2_I$

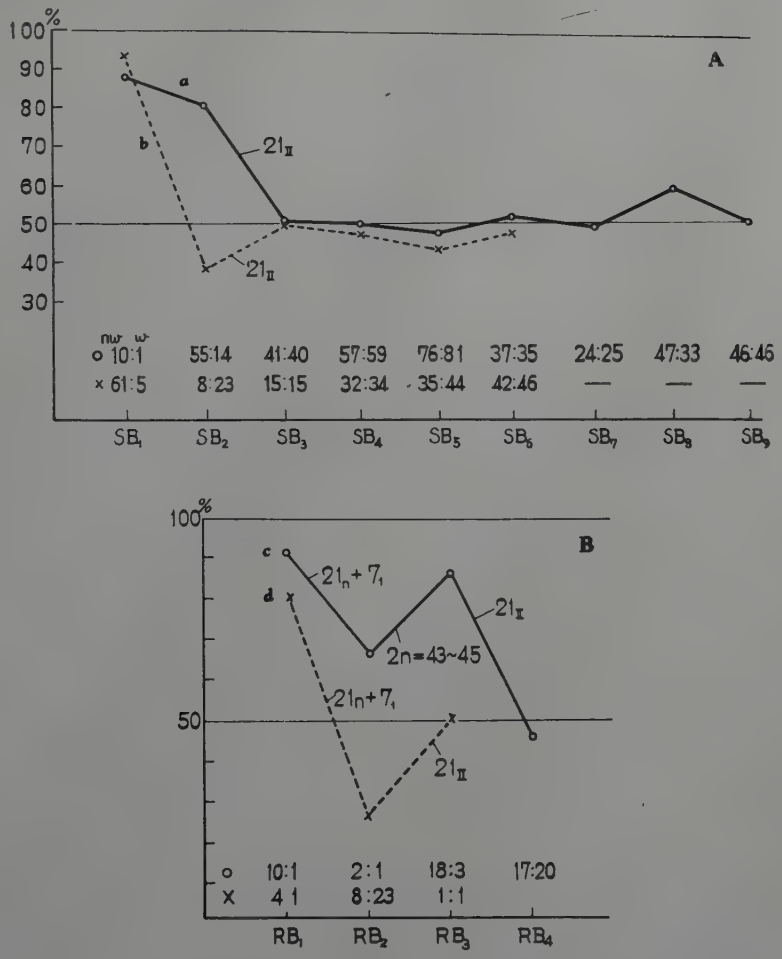


FIGURE 13. Variation in the frequencies of non-waxy plants in B_{1-9}: A, two substitution lines (*a*,*b*); B, two restoration lines (*c*,*d*).

showed a nw > w ratio again as in RB_2 (Fig. B(*c*)). In the next generation which was derived from a 21_{II}-plant, the ratio became 1:1. In this calculation, the ratio was always calculated from the progeny of a single plant in the preceding generation, except for F_1.

The chromosome number of parental generations is indicated by their configuration formulae ($21_{II} + 7_I$, 21_{II}, etc.). After the backcrossed generations have reached the 6*x* level (21_{II}), the chromosome number is not mentioned again. Hereafter, the ratio becomes 1:1. This definitely shows that the gene in question is transferred to the *vulgare*-genomes.

So far no example showing the ratios non-waxy = waxy and non-waxy ⩽ waxy in RB_2 has been mentioned. Though the number of plants was small, such plants were found many times: namely, 8:8, 10:13, 4:8, 4:9, and 2:7 (Table VIII). In RB_4 similar ratios were found in four strains. By selfing them ($n = 21_{II}$) the 3:1 ratio was obtained in the progeny (Table IX). Some discrepancies between the results obtained in RB_4 and $(RB_3)^{S1}$ suggest the possibility of competition between the gametes.

TABLE IX

SEGREGATION OF NON-WAXY AND WAXY IN RB_4 AND $(RB_3)^{S1}$

	RB_4	$(RB_3)^{S1}$
RB_3	nw : w	nw : w
31–2	28 : 36	69 : 23
–3	12 : 20	55 : 11
33–2(21_{II})	—	48 : 25
–4	4 : 5	34 : 10
TOTAL	44 : 61	206 : 69

As to the black-ear character, it appeared very often in B_2-plants with higher chromosome numbers (47–49) in accordance with expectation, and their frequency became lower with the decrease in chromosome number. Usually they disappear when the plants attain the hexaploid level. This shows that there is no crossing-over between the black *caudata*-chromosome and a *vulgare*-chromosome. As has been shown, the chromosome carrying this character has replaced one whole *vulgare*-chromosome. This alien substitution could have been attained by selfing of B_1 or B_2.

Purple stems disappeared very fast, faster than black ear, with the elimination of univalents in the course of backcrossing.

Although the behavior of the remaining *caudata*-characters (late maturity, winter habit, short culm, and profuse tillering) was not studied in detail, it was ascertained that these characters disappeared gradually with the advance of backcross generations. As the height of the plants can be compared easily, a measurement of culms was undertaken in the ninth generation. The height of *T. vulgare* and P 168 was 144.9 cm. ± 1.9 and 140.1 cm. ± 1.9 respectively. Four strains of SB_9 and seven of RB_9 were measured. In general, they were a little shorter than the hexaploid parents. But some RB_9 plants showed no significant difference in height when compared with *T. vulgare* and P 168, while all SB_9 plants were significantly shorter: for example, the height of SB_9 172 was 129.6 cm. ± 4.7.

FERTILITY IN THE BACKCROSSES B_{1-9}

Fertility was estimated mainly on the basis of the percentage of seeds set and the percentage of good pollen. The setting of seeds by self- and open-pollination was also taken into consideration.

To begin with, the results on seed-fertility obtained for F_1, B_1, B_2, B_3, and B_4 will be given. Table X shows seed-fertility of the SB- and the RB-series, each

TABLE X

SEED AND POLLEN FERTILITY IN F_1, B_1, B_2, B_3, AND B_4

		F_1	B_1	B_2	B_3	B_4
Seed	SB	4.4	38.6	55.6	70.8	68.2
	RB	2.7	21.8	77.8	95.0	86.3
Pollen	SB	0.0	38.3	58.1	4.9	0.1
	RB	1.2	45.5	5.5	62.1	93.7

represented by one strain. Fertility of each backcross generation is represented by one individual, from which the progeny was obtained. It is clear from this table that seed fertility is rising to the normal level with successive backcrosses.

There was wide variation in seed fertility in younger B-generations, especially in B_2. Therefore, it is a matter of chance that the curves for the SB- and RB-strains are directed upward. Table XI shows the variation in seed fertility of B_1 and B_2.

The reason why the B_2 is highly variable may be found in the incompleteness of V-genome due to recombination between V (ABD) and C in B_1, though the chromosome number may be equal to $6x$.

TABLE XI

VARIATION IN SEED AND POLLEN FERTILITY IN RB_2

		Fertility				Fertility				Fertility	
RB_1	$(2n)$	Seed	Pollen	RB_2	$(2n)$	Seed	Pollen	RB_3	$(2n)$	Seed	Pollen
10–2 ('54)	(49)	25.0	38.2	16–3	(43)	27.9	5.6				
				–7	(43)	12.5	40.9				
				–9	(43)	51.4	42.9	22–1	(42)	75.0	74.2
12–1 ('55)	(49)	64.0	13.4	15–37	(42)	20.3	0.0				
				–39	(42)	16.6	–0.0				

Pollen fertility is also shown in Table X. It was usually variable even within one individual, especially in the SB-series. In RB_1 one plant was used, whose pollen has been observed twice. RB_2 showed the lowest pollen fertility.

It should be noted that the percentage of good pollen in RB_4 amounted to more than 80 per cent, while SB_4 had almost no good pollen at all. Correspondingly, setting of seeds by self-pollination was high in RB_4 (80 per cent), while SB_4 was completely sterile.[5] A close observation of the pollen grains revealed remarkable differences between SB_4 and RB_4 (Fig. 14).

According to Kihara's method, the pollen grains were classified into five types: I, normal, with one vegetative and two elliptic sperm nuclei; II, normal or almost normal, with one vegetative and two round sperm nuclei; III, binucleate; IV, uni-

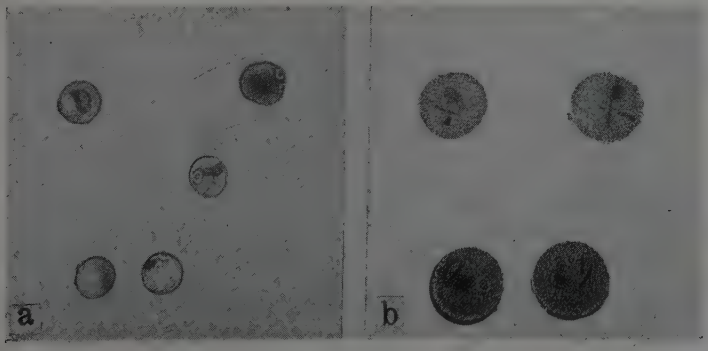


FIGURE 14. Photomicrographs of pollen grains of (*a*) SB_4 and (*b*) RB_4.

[5]In one of many bagged ears a single grain was found; it might have resulted from a technical error.

nucleate; and V, empty. Only type I was considered to be functional. Types II–V were more or less poor in cytoplasmic contents or empty.

In RB_4 the intermediate types (II–IV) are very rare, type I being the most frequently found. On the other hand, there were many intermediate types found in SB_4, and the distribution followed almost exactly the normal curve (Fig. 15). Many mitotic figures in the grains were observed at flowering time.

In 1952 it was found that plants in some RB_3-strains having 21 bivalents had good and bad anthers mixed. The latter were shrivelled and did not shed pollen. Such plants were crossed reciprocally with *T. vulgare* in order to find out if the abnormality was transmitted through the pollen. The results of this experiment are shown in Table XII. It seems very probable that the abnormality is transmitted to a greater extent through the pollen than through the egg cells. By selecting plants with good anthers, we eliminated this character completely after RB_4.

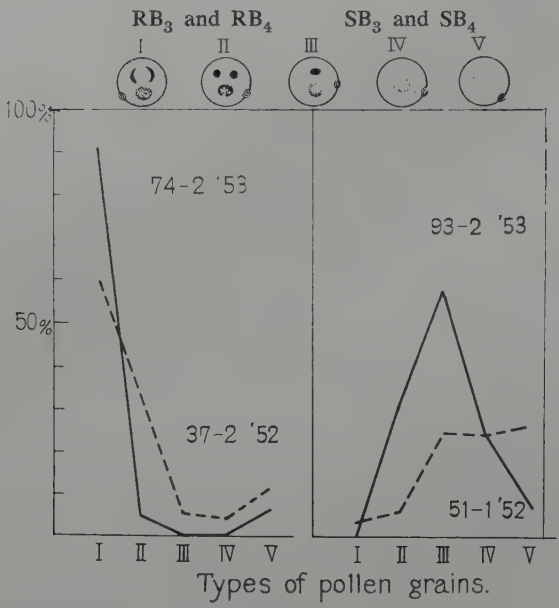


FIGURE 15. Frequency of five types of pollen grains in SB_4 and RB_4.

TABLE XII

CROSSING EXPERIMENTS SHOWING STERILITY TRANSMISSION THROUGH POLLEN

Crosses (1952)	Cult. no. (1953)	Plants having bad anthers mixed with good anthers*	Plants with good anthers only*
♀ ♂			
41–4 × *T. vulgare*	82	3 (9.3)	29 (90.7)
T. vulgare × 41–4	83	8 (72.7)	3 (27.3)
41–4 selfed	192	9 (60.0)	6 (40.0)
37–4 × *T. vulgare*	75	0 (0.0)	13 (100.0)
T. vulgare × 37–4	76	3 (15.7)	16 (84.3)
37–16 × *T. vulgare*	99	0 (0.0)	14 (100.0)
T. vulgare × 37–16	100	7 (50.0)	7 (50.0)

*Figures in parentheses represent percentage.

From B_5 to B_9 seed fertility remained high and constant, though there was some fluctuation due to weather and other technical conditions, especially the transfer to new experimental fields.[6] However, seed fertility was sometimes as high as 95 per cent in both SB- and RB-series. Seed fertility of SB_{1-9} and RB_{1-9} is shown in Figures 16 and 17. Pollen fertility of RB_4–RB_9 is almost constant though some improvement is perceivable, while no good pollen was found in SB_4–SB_9. Therefore, the SB-series gave no seed when the spikes were bagged. Pollen fertility of SB_{1-8} and RB_{1-8} is given in Figures 16 and 17.

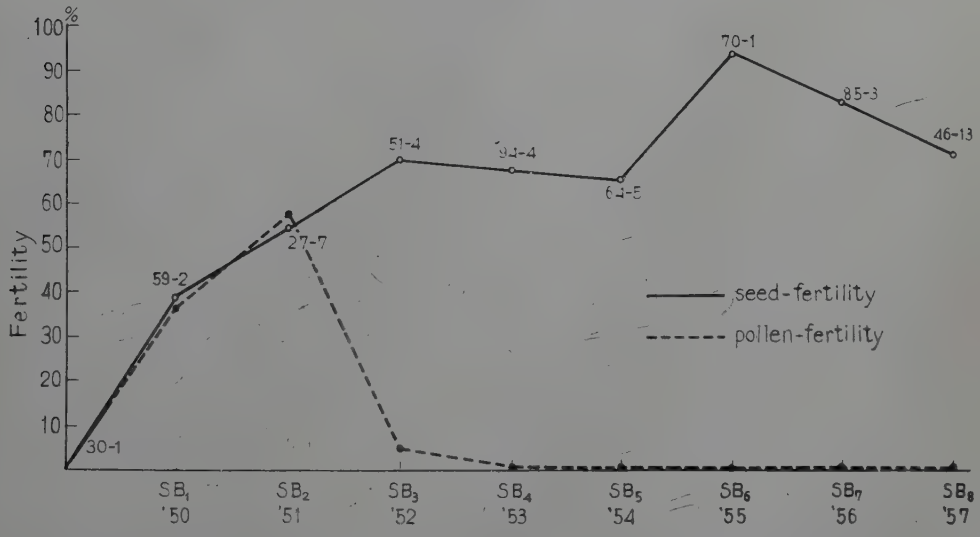


FIGURE 16. Variation in seed and pollen fertility in SB_{1-8} in the course of backcross generations.

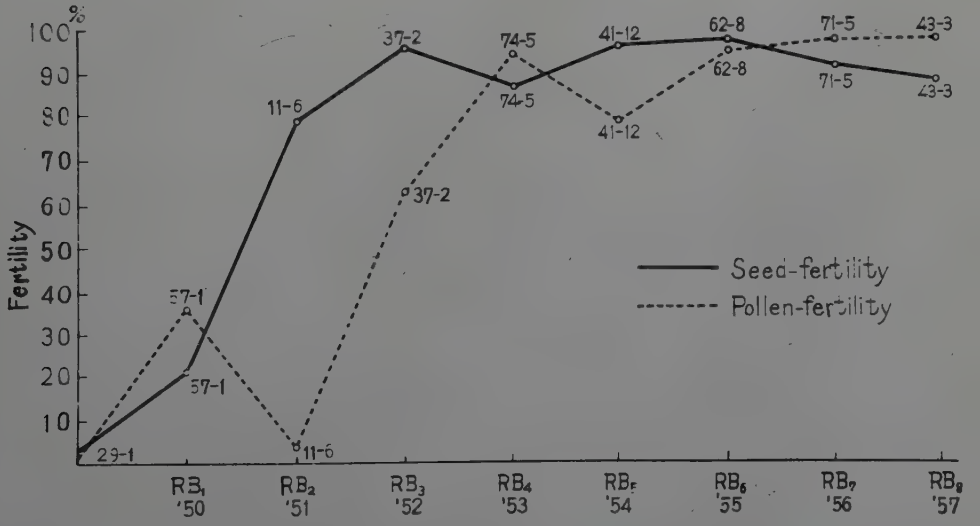


FIGURE 17. Variation in seed and pollen fertility in RB_{1-8} in the course of backcross generations.

[6]The author moved from Kyoto to Misima in 1956.

From time to time RB_{4-9}-plants were selfed in order to examine the degree of restoration. If restoration had been incomplete, variants should have been found (dwarf plants, for example) in the progeny. We could find this year no aberrants in $(RB_8)^S1$.

Morphological Characters associated with *caudata*-chromosomes

Our ultimate aim was to obtain plants which have the whole *vulgare*-genome-complement in *caudata*-plasma in place of *vulgare*-plasma. However, in the course of this procedure, three intermediate products were obtained: (1) addition of *caudata*-chromosomes to *T. vulgare* (VV + x); (2) substitution of a *vulgare*-chromosome by the corresponding *caudata*-chromosome (alien substitution); (3) introduction of *caudata*-genes to *vulgare*-genomes (gene exchange or segmental substitution). As B_1-plants with 49 chromosomes (V'VC') were used for back-crossing, addition of *caudata*-chromosomes to the *vulgare*-genome in B_2 can be simply calculated by the number of chromosomes exceeding 42. Of course, as already indicated, not all of the 42-chromosome B_2-progeny are pure *vulgare*. Some of them may have one or more *caudata*-chromosomes which substituted the corresponding *vulgare*-chromosomes. This makes the analysis more complicated.

In 1951 and 1956 many B_2-plants were obtained. Their chromosome numbers and morphological characteristics were determined as accurately as possible. Morphological records were made for many characters. Four dominant characters were used as markers of *caudata*-chromosomes.

In Table XIII, frequencies of fragile and non-fragile B_2-plants are given, classified according to their chromosome numbers. This table shows that the fragile plants have chromosome numbers varying from 44 to 49. This might mean that at least two *caudata*-chromosomes are necessary to affect this trait. The mean chromosome number (45.7 ± 1.42) of fragile individuals exceeds significantly that of non-fragile ones (44.13 ± 1.28).

TABLE XIII

Chromosome Number and Fragility of Ears in SB_2 and RB_2

		Chromosome number										Total number of plants
		40	41	42	43	44	45	46	47	48	49	
SB_2 (1951)	Fragile	0	0	0	0	2	1	5	1	0	0	9
	Not fragile	0	0	1	13	5	10	4	2	0	0	35
RB_2 (1956)	Fragile	0	0	0	0	4	2	1	2	1	1	11
	Not fragile	0	1	4*	4	11	9	3	0	0	0	32
Total	Fragile	0	0	0	0	6	3	6	3	1	1	20
	Not fragile	0	1	5	17	16	19	7	2	0	0	67

*In one of them the ears were breakable under slight pressure.

In Table XIV the three remaining alternative characters (black/yellow, non-waxy/waxy, and purple/yellow) in relation to chromosome numbers are given. The average chromosome number of all plants showing the dominant characters is higher than that of the recessive individuals. The results agree with the expectation.

TABLE XIV

CHROMOSOME NUMBER AND THREE DOMINANT CHARACTERS OF *caudata* IN SB_2 AND RB_2 (1951 AND 1957)

	Chromosome number										Total number of plants
	40	41	42	43	44	45	46	47	48	49	
Black spikes	0	0	1	7	12	12	10	5	1	1	49
Yellow spikes	0	4	8	27	27	18	8	2	1	0	95
Non-waxy	1	0	7	18	20	28	17	5	1	1	98
Waxy	0	4	3	22	25	7	5	3	1	0	70
Purple stems	0	1	0	6	12	7	6	4	2	1	39
Yellow stems	0	4	9	27	29	25	13	3	0	1	111

However, it will be noted that chromosome-pairing was not constant in many B_2-plants. For instance, 42 chromosomes in B_2 should pair, resulting in 21_{II}, if they are all pure *vulgare*-chromosomes. However, their configuration varies to a certain extent. This indicates that the gametes of such B_1-plants contained either alien substitution or segmental substitution, or both.

Application of the χ-square method to four dominant characters proved that they are all independent. One example is shown in Table XV.

TABLE XV

χ^2-TEST FOR THE INDEPENDENCE OF TWO PAIRS OF *caudata* CHARACTERS

	Fragile	Not fragile	Total
Non-waxy	16	55	71
Waxy	5	11	16
TOTALS	21	66	87

$\chi^2 = 0.54$; P : 0.5–0.25.

The next approach toward the solution of the problem is based upon karyotype analysis. If we knew the kind and the number of additional *caudata*-chromosomes and if we had at the same time detailed data on the morphology of the plants in question, we might guess what characters are associated with C-Sat-1, C-Sat-2, etc.

First, let us take one example, a 43-chromosome RB_2-plant (No. 11-6, 1951) whose karyotype is 2 Sat-1 + 2 Sat-2 + 1 C-Sat-1 beside 38 V- or L-shaped *vulgare*-chromosomes. The karyotype indicates that this plant has the whole *vulgare*-genome assortment and one *caudata*-chromosome (C-Sat-1). Morphologically none of four dominant characters of *caudata* was found in this plant. One 42-chromosome progeny (RB_3) obtained from this RB_2 (11-6) showed the karyotype 2 Sat-1 + 2 Sat-2 + 1 C-Sat-1; chromosome-pairing in PMC was 21_{II} (rarely $20_{II} + 2_I$). One of the ordinary *vulgare*-chromosomes had been replaced by 1 C-Sat-1. Here, too, no pronounced morphological difference between this plant and *T. vulgare* was found.

The other Sat-chromosome, C-Sat-2, behaves quite differently. Dominant genes located on C-Sat-2 can manifest the characteristics which they control either by addition of this chromosome to the *vulgare*-genomes or by substitution of one *vulgare*-chromosome by it. So far we know this chromosome carries three genes. One is a marker gene producing black ears (Kihara and Muramatsu, 1955). The second is a gene for partial restoration of pollen fertility (Kihara, 1951). The third is a gene (or genes) for short culms. In one strain, obtained by open pollination, plants with black and yellow ears were segregated. Measurement of the height showed that the black individuals were 10.5 cm. shorter than the yellow ones.

So far when two i-shaped *caudata*-chromosomes have been added to the *vulgare*-genomes, no *caudata*-characters have appeared. Nor did either of them show any other morphological effect except for some quantitative characters (height, etc.). It seems that the i-shaped chromosomes do not conjugate with the *vulgare*-chromosomes.

A 43-chromosome RB_2-plant, which possessed one *caudata* J_2-chromosome in addition to the *vulgare*-genomes, did not show any alteration of *vulgare*-characters, while ears of two individuals possessing 1 J_2 + 1 i were fragile. Probably co-operation of two chromosomes is necessary for disarticulation of the ear. This suggestion agrees with the result of the chromosome count (Table XIII). I could not identify the i-shaped chromosomes.

The last *caudata*-chromosome, J_1, is not easily distinguished from *vulgare*-chromosomes owing to their morphological similarity. All other *caudata*-chromosomes were known not to relate to the non-waxy character, which is clearly governed by one dominant gene. We might be allowed tentatively to locate non-waxy on this chromosome (J_1). As for purple we have no knowledge of its relation to *caudata*-chromosomes. This character appeared in all of 49-chromosome B_1-plants. Only 24 per cent of B_2 have shown this character. Probably two or more *caudata*-chromosomes (probably i-shaped ones) are necessary for its manifestation.

As the dominant genes of *caudata*-chromosomes in backcross generations might have been transferred previously by crossing-over, the relation between genes and characters cannot be decided with absolute certainty. However, tentative relations are presented in Figure 18.

Observations on Genome Manifestations in Foreign Plasma

The most remarkable difference between *T. vulgare* and RB_9 on one hand and SB_9 on the other is the male sterility of the latter. Male sterility may be attributed to a disharmony between plasma and genomes. In a search for other differences which could be attributed to the relation between plasma and genomes, rust resistance, left- and right-handedness of spikelets, and pollen fertility were observed.

Rust resistance. Inoculation experiments with yellow and brown rust were undertaken. A slight difference in the resistance to yellow rust was found between the two parents: *T. vulgare* was from moderately resistant to susceptible (infection types 2-3), while *Ae. caudata* was very susceptible (type 4). Both SB_9 and RB_9 were susceptible (3-4). For brown rust all materials examined were susceptible.

Left- and right-handedness of spikelets. C-value (a quantitative expression for the regularity of the alternate sequence on the ear of right- and left-handed spikelets (Kojima, 1953)) was calculated for *T. vulgare*, *Ae. caudata*, and several strains of SB_9 and RB_9. The results are shown in Table XVI. In this table the height of the respective plants is also given. C-value of three SB_9-plants, in which the substitution

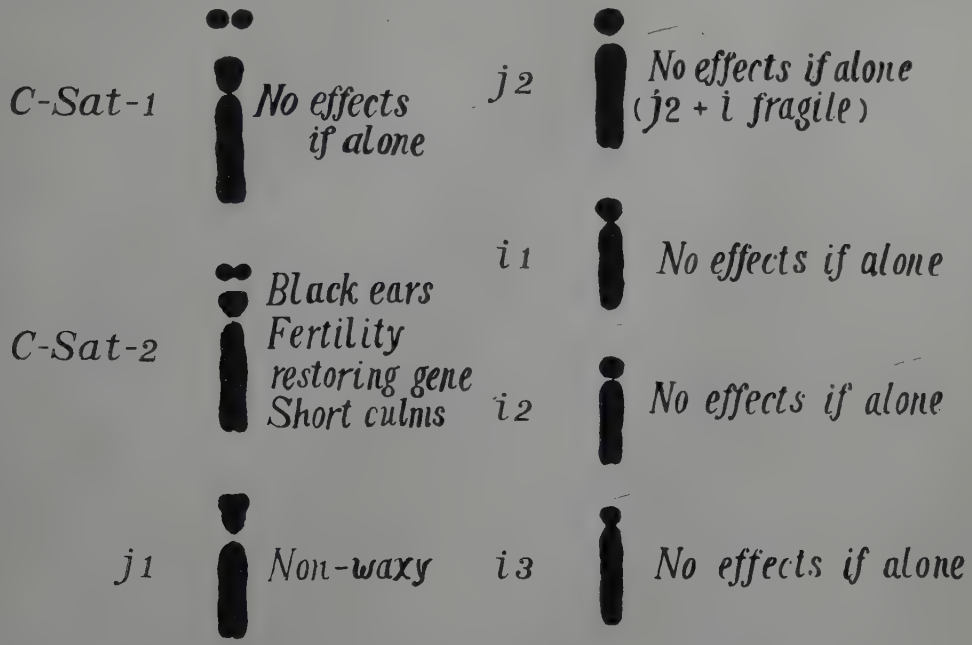


FIGURE 18. Relations between *caudata*-chromosomes and external morphology when substituted or added to *vulgare*-genomes.

TABLE XVI

$\bar{C}$-VALUE AND HEIGHT OF SB_9 AND RB_9

Material	$\bar{C}$	Height (cm.)
T. vulgare	63.6±1.7	144.9±1.9
Ae. caudata	55.2±1.8	49.5±1.1
SB_9 (No. 174)	48.1±2.6	136.7±1.9
SB_9 (No. 175)	49.3±3.3	120.3±5.5
SB_9 (No. 176)	49.1±2.6	135.1±4.1
RB_9 (No. 164)	59.2±2.9	137.9±2.2
RB_9 (No. 166)	61.2±1.7	151.2±1.9
RB_9 (No. 168)	54.6±1.7	137.1±1.8
RB_9 (No. 170)	57.2±1.4	133.6±4.8

of *vulgare*-genomes into *caudata*-plasma might have been almost completed, was significantly different from that of *T. vulgare*. The spikelets of these three SB_9-plants, like those of *Ae. caudata*, showed no antidromic regularity. It seems that the antidromic pattern has some specific relation to plasma. Both $\bar{C}$-value and the height of an RB_9 strain (No. 166) reached to the level of *T. vulgare*.

Pollen fertility of the hybrids. In order to examine the behavior in respect to fertility in combinations between SB_8 and other hexaploids, the following crosses were studied: (*a*) $SB_8 \times$ *T. spelta* was male sterile as SB_9; (*b*) $SB_8 \times$ *T. compactum was* partially male fertile (see fertility by selfing 2.5 per cent); (*c*) SB_8

× *T. macha* died before shooting—the same result was obtained in 1949 with *T. vulgare* and *T. macha* (Kihara, 1949).

Substitution Experiments with Other Cereal Species

Substitution experiments conducted by Japanese geneticists (except Lein) are briefly summarized in Table XVII.

TABLE XVII

Summary of Substitution Experiments in Cereals

Plasma	Nucleus	Male	Female	Author
T. durum	*Ae. ovata*	Fertile	Fertile	Fukasawa (1957a)
Ae. ovata	*T. durum* *T. orientale* *T. persicum* *T. pyramidale* *T. polonicum* *T. dicoccoides* var. *spontaneo-nigrum*	Sterile	Fertile	Fukasawa (1957b)
Ae. ovata	*T. dicoccoides* var. *Kotschyanum*	Fertile	Fertile	Fukasawa (1957b)
T. vulgare	*Secale cereale*	Fertile	Fertile	Lein (1948) Sasaki (1952)
Ae. longissima	*Ae. Aucheri*	Weakly fertile (SB_6)	Weakly fertile	Kihara (1951)
Ae. speltoides	*Ae. umbellulata*	Lethal		
T. Timopheevi	*T. dicoccum*	Fertile	Fertile	Kihara (unpublished)

General Conclusions and Discussion

Substitution of Whole Nucleus

According to Kimura (1950), the speed of substitution depends on the number of haploid chromosomes and percentage of crossing-over. His calculation was made on the following assumptions. (1) Two parental species have the same chromosome number. The homologous chromosomes conjugate with each other. For each pair 30 per cent crossing-over is assumed. (2) The fertility of individuals with different genetic constitutions is the same. In our material Kimura's F_1 (0th generation) corresponds roughly to B_2 in SB-series and B_3 in RB-series, when the chromosome number has become constant (21 bivalents).

Using Kimura's formula we obtain the frequency percentage of individuals having no substituted segments from 0th to 10th generation. If we assume further that seven pairs out of 21 bivalents are already homologous in the 0th generation, m is equal to 14, as given in Figure 19. This theoretical calculation is made on the basis that the backcrosses are made at random. In our experiments strong selection was carried out for complete substitution or restoration. Therefore, it is highly probable that some of SB_9- and RB_9-plants might already have pure *vulgare*-chromosomes. This is shown by their karyotypes, fertility, and morphology.

Male sterility is one of the aspects subjected to substitution. The meiosis in male-sterile lines (SB_4–SB_9) is normal. But the microsporogenesis is so retarded that almost no pollen grains are found to develop normally. The co-operation between plasma and genomes seems to be insufficient in pollen grains, possibly owing to the unsatisfied need for a rapid supply of nuclear substance. So far, no visible differences have been found between the tapetum cells of SB_4 and RB_4.

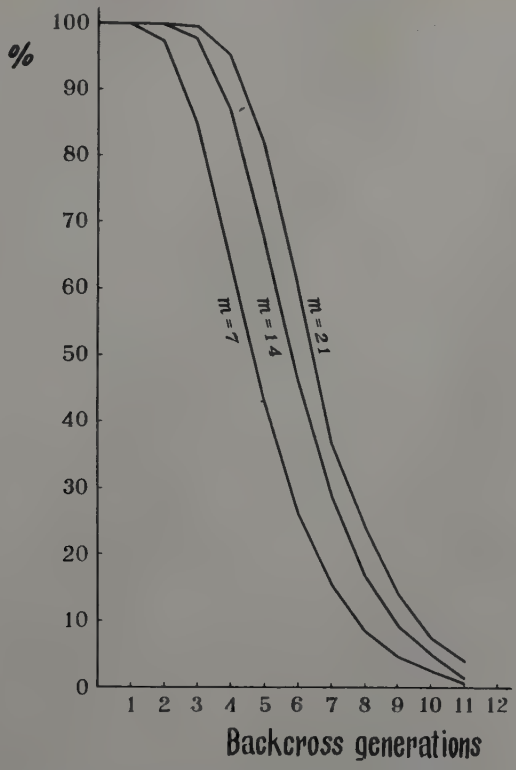


FIGURE 19. Frequency percentage of individuals (ordinate) with non-substituted chromosome segment in successive generations. In each chromosome pair 30 per cent crossing-over is assumed. Abscissa: number of backcrossings; *m*: number of chromosome pairs.

Similar male sterility was artificially induced by Fukasawa *et al.* (1957) by keeping water-cultured ears in a dark room. He observed in his male-sterile strain (genomes of *T. durum* in plasma of *Ae. ovata*) that asparagine was accumulated, while proline was lacking (Fukasawa, 1954).

Chromosome Substitution

As to the number of chromosomes, all B_3 have attained the 6*x* level in our experiments. This does not guarantee complete genome restoration or substitution. As substitution of *caudata*-chromosomes and crossing-over between *caudata*- and *vulgare*-chromosomes might have taken place in preceding generations, we had to restore their purity by further successive backcrosses.

We do not know yet how the substitution of alien chromosomes takes place in B_1-gametes. However, we can guess that substitution could be possible by frequent illegitimate pairing in 49-chromosome B_1-plants ($21_{II} + 7_I$). Suppose one of 21_{II} of this B_1 has a pair consisting of two chromosomes, say Sat-1 and C-Sat-1, while the other Sat-1 remains univalent. If we backcross to *vulgare*, we should expect plants in B_2 which have in their complement C-Sat-1 instead of Sat-1. Now we have to go back to the chromosome association in F_1 observed in the post-contraction

stage which has the appearance of diakinesis. There were 14 side-by-side or end-to-end associated bivalents in one PMC. Although conjugation of all chromosomes was rather rare, there were undoubtedly more paired chromosomes at this stage than at MI.

Recent findings in true haploid and polyploid plants show that semi-homologous and even non-homologous chromosomes tend to associate with each other at prophase until a pairing tendency of unknown nature is saturated (Rieger, 1957; Riley, 1957). In our experiments i-shaped chromosomes (especially two smaller ones) do not pair either with each other or with *vulgare*-chromosomes at MI either in B_1 or in the following generations. However, in Figure 11c, we can recognize at 5 o'clock a pair consisting of two i-shaped chromosomes (most probably i_2 and i_3). The i_1, the largest among the three i-shaped chromosomes, seems to lie beside a chromosome with a subterminal fibre attachment (at 8 o'clock, Fig. 11c). They lie head to tail. Such attachment of chromosomes (should it be maintained until MI) might explain why we found 22 or more bivalents in $(RB_1)^{S1}$ and RB_2 (see p. 146).

We do not know yet whether this apparently non-homologous pairing is only a so-called secondary association of two chromosomes or if they already have gone through the same process of eventual exchange of genetic materials to which homologous pairs are subjected. However, there must be some kind of transfer of genetic material from *caudata* to *vulgare*. If so, conjugation and crossing-over are not the monopoly of truly homologous chromosomes.

Theoretically, there may be a gradual transition between truly homologous and non-homologous chromosomes. Roughly they can be divided into three groups: (*a*) true homologous, (*b*) semi-homologous, and (*c*) non-homologous. Bivalents resulting from homologous pairing retain their association until metaphase, while only some of the semi-homologous bivalents are kept together and the non-homologous pairs become dissociated and form univalents at MI.

These speculations encounter difficulties since semi-homologous pairs behave like normal bivalents, as several chromosome substitutions have shown. The C-Sat-2 substitution might be the best example. When this chromosome is incorporated into the *vulgare*-genome in a single dose, the semi-homologous pair in which it is involved behaves quite normally. For this kind of conjugation I should like to propose the term facultative conjugation. But this suggestion leads to the conclusion that the behavior of a semi-homologous pair is subjected to genotypic control.[7] On this point the question could be raised whether C-Sat-2 might have been changed by repeated association with the corresponding *vulgare*-chromosome in successive backcrosses. However, C-Sat-1, another Sat-chromosome of *caudata*, behaves quite regularly when it is transferred as a single unit into the *vulgare*-genome (RB_2 37-2, Table VII).

This relation between C-Sat-2 and a *vulgare*-chromosome (Sat-2 or a non-Sat-chromosome) might be similar to the semi-homologous relations between the three wheat genomes, A, B, and D. As a result of Sear's investigation, seven groups of what he calls homeologous chromosomes have been established. Each group consists of three chromosomes, each belonging to one of the three genomes, A, B, and D.

Gene or Segmental Substitution

We have assumed that the non-waxy gene is transmitted to *vulgare*-genome by crossing-over. Transfer of a gene or genes from alien chromosomes is not very rare

[7]On the other hand, two homologous chromosomes (like the two C-Sat-2 observed in our F_1 between P 174 and *Ae. caudata*) cannot maintain their obligatory association and become desynaptic owing to their hemizygous condition.

in plants. I have very often found 49-chromosome B_1-plants which might have had a double dose or none of non-waxy genes. As already explained their occurrence was attributed to crossing-over in F_1-gametogenesis. We have found four B_1-plants which might have possessed two non-waxy genes resulting in the segregation ratio of nw>w in B_2. The total number of B_1 was 18 including those whose B_2-offspring were not investigated (Table VIII). Accordingly, their occurrence is very frequent.

At the beginning of this experiment, the difference in segregating ratios was ascribed to the reciprocal crosses, but the results, summed up in Table VIII, make it clear that there is no difference between reciprocal crosses (see segregation ratio nw : w in Table VIII).

In RB_4 the pollen grains develop quite well. But in some plants shrivelled anthers often occur mixed with good ones; they contain many empty pollen grains (around 73 per cent). This kind of pollen sterility was to a larger extent transmissible through the pollen than through the egg cells (Table XII). Therefore, we may assume that the *vulgare*-genomes have become, in the previous generations, contaminated with *caudata*-genes or with one or more *caudata*-chromosomes. Three RB_3-plants were involved in this experiment, namely, 37-2, 37-4, and 41-4. The last plant (41-4) was karyotypically identical with *vulgare*, while the first one (37-2) had C-Sat-1 among 42 chromosomes, the remaining 41 supposedly being all *vulgare*-chromosomes. The karyotype of 37-4 is unknown. However, this plant had 21_{II} like the other two. The chromosomes were sticky which would indicate an alteration of the genomes. This kind of sterility is certainly not a result of plasmatic sterility or plasmatic change.

Karyotype Analysis and Morphology

The karyotype of *T. vulgare* was designated by 2 Sat-1 + 2 Sat-2, leaving 38 chromosomes out of consideration. *Ae. caudata* has 1 C-Sat-1 + 1 C-Sat-2 + 1 J_1 + 1 J_2 + 1 i_1 + 1 i_2 + 1 i_3 in the haploid complement. Three i-shaped chromosomes are indistinguishable when only one or two of them are found. In B_1 and B_2 fragments and also new chromosome types were observed which have not been found in the parents.

The karyotype analysis indicated that the addition of individual *caudata*-chromosomes in single dose to wheat was possible for C-Sat-1, J_2, and one or two of three i-chromosomes. None of these *caudata*-chromosomes produced in wheat any of the four dominant characters. Presumably the dominant characters are produced by the complementary action of two or more *caudata*-chromosomes; when one only is present, no complementary genes in the *vulgare* complement seem to be available. One exception is C-Sat-2. If C-Sat-2 is present, the ear of the plant becomes black. In two parental strains, P 168 and P 174, C-Sat-2 replaced one of the *vulgare*-chromosomes. It carries a gene for black ears and a gene which restores fertility. Strong penetrance of these genes reminds us of the "hairy neck" of *Secale cereale* reported by O'Mara (1951) and others.

As indicated in the tables showing the frequencies of alternative characters in relation to chromosome numbers (Tables XIII and XIV), we can conclude that dominant characters are found among B_2-plants with higher chromosome numbers.

Plasma Constancy

As to the color mosaics of ears described in the previous paper (1951), they cannot be considered as due to the influence of plasma upon genes since they appeared reciprocally. So far, no segregation of hereditary plasma units could be observed.

After many years of observation of *vulgare*-plants with *caudata*-plasma ($a^cV^bV^b$, a^cV^bV and a^cVV) it is assumed that plasma and genome independently maintain their individuality though they interact with each other in metabolic activities.

Summary

Triticum vulgare (= *T. aestivum*, $2n = 42$) and *Aegilops caudata* ($2n = 14$) were used in the present investigations. There were no differences in the behavior of chromosomes in the reciprocal hybrids between the two species. None to six bivalents (sometimes more) and up to one trivalent were observed. Often restitution nuclei were found which were expected to give rise to functional unreduced gametes.

Substitution backcrosses with the pollen of *T. vulgare* were carried out to the ninth generation. The F_1-hybrid used for substitution was *Ae. caudata* (female) × *T. vulgare* (male). The backcrosses were carried out according to the following plan:

$$\begin{aligned} F_1 \times T.\ vulgare &= SB_1 \\ SB_1 \times T.\ vulgare &= SB_2 \\ \cdot \quad\quad \cdot &\quad\quad \cdot \\ \cdot \quad\quad \cdot &\quad\quad \cdot \\ \cdot \quad\quad \cdot &\quad\quad \cdot \\ SB_8 \times T.\ vulgare &= SB_9 \end{aligned}$$

Restoration backcrosses with the pollen of *T. vulgare* were carried out to the ninth generation. The F_1-hybrid was *T. vulgare* (female) × *Ae. caudata* (male). The experiment was carried out according to the following schedule:

$$\begin{aligned} F_1 \times T.\ vulgare &= RB_1 \\ RB_1 \times T.\ vulgare &= RB_2 \\ \cdot \quad\quad \cdot &\quad\quad \cdot \\ \cdot \quad\quad \cdot &\quad\quad \cdot \\ \cdot \quad\quad \cdot &\quad\quad \cdot \\ RB_8 \times T.\ vulgare &= RB_9 \end{aligned}$$

Substitution and restoration backcrosses were repeated with several strains of *T. vulgare* and one strain of *Ae. caudata.*

Chromosome numbers in SB_1 and RB_1 varied from 36 to 52, but 49-chromosome B_1-plants were most frequent. Both 49-chromosome SB_1- and RB_1-plants had $21_{II} + 7_I$. The bivalent number varied from 19 to 21. Up to three trivalents were found. To obtain B_2-plants only 49-chromosome B_1-plants were used. In B_2-generation offspring with 40 to 49 chromosomes were obtained. To obtain B_3-plants, 42-chromosome B_2-plants or plants with about that chromosome number were used. In B_3 and the following generations only plants having 21_{II} were used in further backcrosses. Therefore, the chromosome number in later generations became constant with 21_{II}.

T. vulgare is characterized by two sets of Sat-chromosomes, namely, Sat-1 and Sat-2. *Ae. caudata* has in the haploid complement two Sat-chromosomes (C-Sat-1 and C-Sat-2), two J (J_1 and J_2), and three i-shaped chromosomes. F_1 hybrids between P 168 (a *vulgare* strain having C-Sat-2 substituted for one *vulgare*-chromosome) and *Ae. caudata* possessed the whole haploid complement of *caudata* and 1 Sat-1, 1 Sat-2 and 1 C-Sat-2 besides 18 non-Sats of *vulgare*.

B_2- and B_3-plants, which had 21_{II}, were not always karyotypically identical with *vulgare*. For instance, one *vulgare*-chromosome was substituted by 1 C-Sat-1 in RB_3 (37-2).

The pairing in the RB_3-plants (37-2 and 37-4) indicates that C-Sat-1 conjugates either with Sat-1 or a non-Sat-chromosome.

REFERENCES

CÂMARA, A. 1943. Estudo comparativo de cariotipos no género *Triticum*. Agron. Lusitana *5*(2): 95–117.

FUKASAWA, H. 1954. On the free amino acids in anthers of male-sterile wheat and maize. Jap. J. Genet. *29*(4): 135–137.

——— 1957a. Studies on restoration and substitution of nucleus (genome) in *Aegilotricum*. IV. Genome exchange between *durum* and *ovata* cytoplasm and its theoretical consideration for male-sterility. Cytologia *22*(1): 30–39.

——— 1957b. Studies on restoration and substitution of nucleus (genome) in *Aegilotricum*. V. Critical evidence for uneffectiveness of alloplasm on hybridization. Jap. J. Genet. *32*(9–10): 269–276.

FUKASAWA, H., K. MITO, and M. FUJIWARA. 1957. Preventative effect of sugars on the pollen degeneration of wheat plant. Bot. Mag., Tokyo *70* (831): 251–257.

KIHARA, H. 1949. Genomanalyse bei *Triticum* und *Aegilops*. IX. Systematischer Aufbau der Gattung *Aegilops* auf genomanalytischer Grundlage. Cytologia *14*(3–4): 135–144.

——— 1951. Substitution of nucleus and its effects on genome manifestations. Cytologia *16*(2): 177–193.

——— 1954. Restoration and substitution of nucleus. Caryologia Suppl. Vol.: 900–902.

KIHARA, H., and N. KONDO. 1943. Studies on amphidiploids of *Aegilops caudata* × *Ae. umbellulata* induced by colchicine. Seiken Zihô, Rep. Kihara Inst. Biol. Res. *2*: 24–42.

KIHARA, H., and M. MURAMATSU. 1955. A supernumerary trabant chromosome in one substitution wheat strain and its manifestation. I. Jap. J. Genet. *30*(4): 173.

KIMURA, M. 1950. The theory of the chromosome substitution between two different species. Cytologia *15*(3–4): 281–294.

KOJIMA, K. 1953. Studies on the right- and left-handedness of spikelets in Einkorn wheats. III. Concordance proportion, mean concordance proportion and their relation. Proc. Japan Acad. *29*(10): 576–580.

LEIN, A. 1948. Ueber alloplasmatische Roggen. Der Züchter *19* (1/3): 101–108.

O'MARA, J. G. 1951. Cytogenetic studies on *Triticale*. II. The kinds of intergeneric chromosome addition. Cytologia *16*(3): 225–232.

RIEGER, R. 1957. Inhomologenpaarung und Meioseablauf bei haploiden Formen von *Antirrhinum majus* L. Chromosoma *9*: 1–38.

RILEY, R., and V. CHAPMAN. 1957. Haploids and polyhaploids in *Aegilops* and *Triticum*. Heredity *11*(2): 195–207.

SASAKI, M. 1952. A consideration of alloplasmatic rye from the view point of plant breeding. Jap. J. Breeding *2*(2) Suppl. Vol. 7.

SENJANINOVA-KORCZAGINA, M. 1932. Karyo-systematical investigation of the genus *Aegilops* L. Bull. Appl. Bot. Genet. Plant Breed., Series II.

BASIC RESEARCH IN PLANT AND ANIMAL IMPROVEMENT

Donald F. Jones[1]

HETEROSIS, the stimulating effect of crossing related organisms, was known in ancient times and was described in scientific journals in the eighteenth century. But it was not until Darwin's time that it was recognized as having biological importance. After writing the *Origin of Species*, Darwin turned to domesticated animals for evidence of change by selection. In this study he learned the importance of inbreeding in fixing the desirable characters in breeds of livestock. These named breeds had originated in England and on the continent of Europe in the century before his time. Animal-breeders, recognizing the value of close mating, also warned against the dangers of inbreeding. Their conclusions were somewhat contradictory and confusing.

In his characteristic, thorough manner Darwin set out to explore the effects of cross- and self-fertilization, choosing plants rather than animals because fertilization was easily controlled, large numbers could be grown, and successive generations followed annually in most plants. These investigations covered a period of ten years and were reported in a book published in 1876.

Nearly all species of plants worked with were benefited in some degree by cross-fertilization, and were reduced by self-fertilization. Darwin thought that continued inbreeding eventually ended in extinction, as indeed it often does.

One of the plants used by Darwin in his experiments was Indian corn, maize from the New World. These experiments were known to Professor Asa Gray, at Harvard, and through him to one of his students, James Beal, who was soon to experiment and to teach at the first state agricultural college in the United States at Michigan.

Since corn is pollinated by the wind and the flowers are separated in different inflorescences on the same plant, Beal conceived the idea that it could be easily cross-fertilized on a large scale. On the Michigan State College farm the first crossed corn was grown by pulling out the tassels, the male inflorescences, before pollen was shed from all plants used as the seed parents. Pollen was furnished by a different variety used as the pollen parent. For his crosses Beal followed Darwin's advice and selected varieties that had been grown for a period of years under widely different environmental conditions. These varieties also differed in genotype.

From these crossed plants Beal obtained significant increases in yield. The largest increases came from crosses of flint and dent varieties, one grown in the northern, the other in the southern part of the state. The crossed plants, in maturity, suited very well the central part of the state where the trials were made. Beal was so impressed by the sturdy growth and good grain-yields of his first generation crossed plants that he advocated this method of producing seed at farmers' meetings and in the agricultural press.

For various reasons this method was never used commercially. Farmers, seed-producers, and experimental agencies continued to employ the conventional methods

[1]The Connecticut Agricultural Experiment Station, New Haven, Conn., U.S.A.

of selection long used by animal-breeders but made little progress with corn, largely because they had no control over the inheritance from the male parent.

Beal was also interested in following up Darwin's experiments on the effects of inbreeding. He devised a method of covering the flowers of the corn plant with bags and applied the pollen to the plant's own pistillate flowers at the right time to obtain completely self-fertilized seed. In turn, Beal's student, Perry Holden, continued these studies on the effects of inbreeding at the Illinois Experiment Station up to about 1900. These experiments were reported by Shamel in the *Yearbook of the U.S. Department of Agriculture* for 1905. Continued inbreeding was very disastrous to the size and seed-production of the corn plants Holden worked with. Most of the inbred plants were unable to survive more than three generations. As a result of these experiments the Illinois station published bulletins on methods to avoid the undesirable results of inbreeding.

Shortly after the rediscovery of Mendelism, George Shull at the Carnegie Institution in Cold Spring Harbor on Long Island, New York, began a study of the inheritance of a quantitatively variable character. He selected the number of kernel rows on the ear of maize for this study. Maize has from eight to thirty or more rows of grain on the ear. Different varieties fluctuate around a mean number of rows that may differ widely. Shull selected ears of different row number from a field of white corn of one local variety. In order to reduce the variability of his parental stocks each ear-row line was self-fertilized. As expected, the first generation self-fertilized plants were somewhat reduced in size and vigor and each progeny varied around its own mean row number. There were large differences between different progenies all from the same variety. Several of these inbred lines that differed in row number were crossed after one selfing. Shull was surprised at the large increase in vigor and in productiveness of these first-generation crosses of somewhat inbred plants. He started the experiment with no thought of improving the yield of corn, but he saw in his experimental plantings a new method of controlling heredity to obtain better corn plants with enhanced yielding ability. Shull was getting the same results with a cross-fertilized plant that Johannsen had obtained shortly before in the self-fertilized bean plant. He was simply sorting out different genetic lines as Johannsen had done in beans, and Hansen before him in yeast, but in addition Shull's plants were highly heterozygous before inbreeding. It was shown later that these self-fertilized lines could be reduced to uniformity and constancy, and separated into different genotypes by progeny-testing. Since his corn plants were weak and low in productivity in the inbred condition, Shull proposed that they be crossed each generation to obtain maximum growth and yield. Crossing was easily done with maize by pulling out tassels before pollen was shed from plants grown in a seed-producing field, as Beal had done thirty years previously.

This novel idea was developed further by Shull in several articles in the *Proceedings of the American Breeders Association* and received much attention from agronomists and plant-breeders in all parts of the world. Shull did much more than Beal or Holden had done. He showed why it was important to inbreed first, to control and fix superior germplasm, before crossing, to obtain maximum vigor.

In the meantime, a student of Holden at the Illinois College of Agriculture, Edward East,[2] also began an experiment on the effects of inbreeding. He self-

[2]Holden in a letter stated that East was one of his students. I never heard East mention Holden as one of his teachers at the University of Illinois, although he did mention Hopkins and Burrill.

fertilized corn plants by Beal's method of using paper bags in the same year, 1905, that Shull self-fertilized his plants. East, who had graduated as a chemist at the University of Illinois, was employed at the Illinois Experiment Station to analyze ears of corn for protein and oil in an experiment to improve the commercial and feeding value of corn. In this selection experiment, designed and carried out under the direction of Cyril G. Hopkins, head of the Department of Soils and Agronomy, naturally pollinated ears of Burr White corn were analyzed and selected for high and low protein, and high and low oil in the kernels. After this experiment had been underway for several generations, it was noted that the yields, compared to the original unselected variety, were decreasing.

East studied the pedigrees of the selected lines and noted that all of the high-protein selections traced back to one plant at the start. He recognized that they were getting an indirect inbreeding effect by their close selection. He then proposed to Hopkins that they should make a further, more direct study of inbreeding. Hopkins was not interested in this, as Beal, Holden, and Shamel had already shown that inbreeding was detrimental to corn. Fortunately, on his own initiative and without the approval of his chief, East started an inbreeding experiment of his own.

For his material East selected a different variety, a high-yielding strain of yellow dent corn known as Chester's Leaming. Yellow corn had been recognized as having higher feeding value than white corn and mid-western farmers were rapidly changing from the white to the yellow grain in spite of the fact that the experiment station chemists could find no differences at that time. All of the Illinois inbreeding and selection experiments, up to that time, had been made with white corn, using a variety known as Burr White.

When federal funds for fundamental research became available to the state experimental stations, East left Illinois to work at the Connecticut Agricultural Experiment Station at New Haven (September 1905). He left before his hand-pollinated, self-fertilized ears of Leaming were mature. He asked his colleague, H. H. Love, to harvest these for him and send them to New Haven when they were properly matured. These self-fertilized lines have been grown and self-fertilized continuously in Connecticut to the present time. Three of them, out of sixteen or more original lines, have survived and have now been self-fertilized fifty generations.

When Shull presented his classical report to the American Breeders Association meeting in Washington, D.C., in January 1908, under the title, "The Composition of a Field of Maize," East was in the audience and recognized immediately the importance of Shull's findings. By this time East's inbred lines had been selfed twice, but no intercrosses had been grown. However, a few crosses of these inbred lines had been made in 1907. These were grown in 1908 and in the following years. Crosses were also made between various inbred lines out of white and yellow dent, flint, pop, sweet, and floury maize. Some of these crosses were made between inbreds out of the same varieties. East confirmed Shull's findings and showed that very high yields could be obtained from crosses of certain inbreds from different varieties of corn. All of Shull's inbreds were out of one variety.

Naturally, there was much interest in this revolutionary new method of corn-breeding that promised so much from a theoretical viewpoint: first, control over the heredity from both the male and female parents; and second, maximum hybrid vigor. The first crosses of inbreds which had become adapted to eastern conditions failed miserably in the mid-west and did not convince western corn-breeders that this method was practicable. Moreover, both East and his assistant and later successor at the Connecticut Station, H. K. Hayes, found that there were serious handicaps

in the utilization of first-generation crosses of selfed lines. The inbred plants were so small and weak that seed yields were low and the kernels produced on inbred plants, although cross-fertilized, were so small and poorly shaped that they could not be handled in farmers' planting machines, and the young seedlings started with a handicap compared with large well-nourished seeds from a vigorous plant. After repeated trials both East and Hayes gave up trying to use crosses of inbreds for commercial production and reverted to Beal's method of making varietal crosses.

One further step was needed. By combining four inbreds in two successive crosses in what I have called a "Double Cross," all the handicaps were overcome, maximum hybrid vigor was not only maintained but actually increased because more diverse germplasms could be brought together. A further advantage was added in genetic homeostasis, a factor not fully recognized at the time but now more generally appreciated. The variability of the double cross as compared with the germinal uniformity of the single cross, at first considered to be a serious objection, is of great importance in giving wide adaptability to corn hybrids. The double-cross method is now being used with many cross-fertilized plants and with animals. It also has great promise for forest trees and in a modified form can be used to advantage with self-fertilized crops to give an initial heterozygosity and heterosis that is perpetuated by self-fertilization as long as possible.

While seedsmen and corn-growers were slow to recognize the values in hybrid corn, in time superior germplasm was found in a relatively few highly selected inbreds, as I predicted in a paper in 1920: ". . . the first-generation cross between inbred strains is handicapped at the outset of its growth. A means is at hand to overcome this. Even if many strains are found which give no appreciable increase in yield over the original variety, this does not vitiate the value of this method of selection, if sometime and somehow superb germplasm can be isolated which will give greatly increased yields. . . ." After thorough testing in many different combinations, the most successful double crosses are now being grown over very wide areas in the United States and other countries. Some varieties are being grown from Maine to Virginia and west to the Missouri River; others produce well in the coastal plains of the Carolinas, the San Joaquin Valley of California, the irrigated valleys of Spain, Italy, Israel, and India.

Thus, basic knowledge of genetics contributed by Darwin, Johannsen, Shull, and East, plus a small invention of my own, the multiple cross, has made hybrid corn possible where the practical farmer, seedsmen, and many agricultural researchers did not succeed. These practical men did not succeed and possibly never would have succeeded without a knowledge of basic genetic principles. It is proof of the adage that a "practical man is often one who continues to practise the errors of his forefathers."

In the United States, where we formerly planted from 100 to 120 million acres of corn each year, we now plant less than 85 million acres and produce regularly more corn than ever. One of our serious problems now is over-production.

Not only has basic research revolutionized corn-production, but it has shown the seedsmen how to eliminate the cost of detasseling by utilizing a new principle of cytoplasmic pollen-abortion with restoring genes. This new method not only reduces costs but also eliminates the serious problem of finding adequate help to pull out tassels which has to be done in all kinds of weather and sometimes in areas where little labor of this type is available. By this new method of emasculation more seed is produced from the same genetic type of seed-parent plants and the farmer obtains still larger yields.

This method of using a sterile seed parent with or without restoring genes in the pollinator is being extended rapidly to many other crops in many different plant families. Other methods of utilizing biological information are available to be used in cross- and self-fertilized plants. I refer to the ring chromosomes as exemplified in *Oenothera* and apomixis as employed by the dandelion (*Taraxacum*) and many grasses. Nature uses these methods to obtain heterosis in self-fertilized plants. Why should we not use the same methods? The principles involved in cytoplasm and gene interaction, I believe, also help us to understand speciation. Thus basic research has made possible increased production and applied research has helped to clarify one of the most elusive problems in the whole realm of biology.

REFERENCES

DARWIN, CHARLES. 1876. The Effects of Cross and Self Fertilization in the Vegetable Kingdom. New York: D. Appleton & Co. Pp. 482.

JONES, DONALD F. 1920. Selection in self fertilized lines as the basis for corn improvement. J. Am. Soc. Agron. *12*: 77–100.

SHULL, G. H. 1908. The composition of a field of maize. Rep. Am. Breeders' Ass. *4*: 296–301.

——— 1909. A pure-line method in corn breeding. Rep. Am. Breeders' Ass. *5*: 51–59.

——— 1910. Hybridization methods in corn breeding. Am. Breeders' Mag. *1*: 98–107.

GENETICS IN SILVICULTURE

C. Syrach Larsen[1]

To find the oldest and largest living plant specimens in the world today we must turn to the trees. On the North American continent there are California's wonderful sequoias, but they do not surpass in age the more modest *Pinus aristata* on which Professor E. Schulman has counted about 4,200 annual rings. In my country, Denmark, there are forests where oaks dating from the time of the Viking raids are still standing. Even though trees of such great age are exceptional, it is customary in silviculture to count on fifty to a hundred years as a "harvest" period. Most of the trees today forming the basis of Canada's wealth in timber and pulp are one hundred or more years old. However, of recent years, under subtropical conditions and using the so-called southern pines and other softwoods, it has been possible to obtain the desired yield after twenty to thirty years. It is inspiring to a forester to see an eight-year-old, eighty-foot stand of eucalyptus, ripe for felling.

The time it takes for one tree to grow to maturity is only one of the difficulties which make the work of dendrologists difficult. Another one is that trees have a tendency to bear their flowers high up, inaccessible to close observation. Then too, a tree is large and not easily brought home for study. Moreover, in silviculture it is customary to use a large number of plants per unit of area in sowing or planting. Subsequent thinnings at different ages gradually reduce the number until in the mature stand there are only a few of the original specimens left. The object of such selective thinning is to give greater space to a lesser number of trees, to cut out the poorest individuals, and to stimulate the growth of the best, whether the latter are promising owing to inherited characteristics, or on account of good conditions for growth. If timber prices are high, even small, poorly developed specimens, when thinned out, will have a market; at other times and in other countries thinning may be an economic burden. Under all circumstances it seems improbable that thinnings, so necessary for adequate silviculture, can be mechanized.

It is not surprising then that few people have been tempted to apply classical methods of plant breeding to silviculture. On the other hand, let us admit that those very specimens which require twenty to a hundred years for maturing will derive particular benefit from breeding improvements. All advantageous characteristics inherent in seed act gratuitously for a long term of years, whereas technical devices for improvement are a constant expense.

Even though silviculture affords possibilities for improving the form of the trees by reciprocal placement during a long life, it is obviously reasonable to try to produce specimens with desirable hereditary characters for greater yield when harvested or felled. Until recently in many part of the world, there has been such a wealth of timber that no thought was given either to sowing or to planting. This is particularly true in the northern hemisphere with its large, homogeneous forests of softwood. The increasing demand for wood over the past few decades is changing this situation. Even though a large part of the demand is for pulp for newspapers and prefabricated articles, there is also a demand for wood of finer quality. We must

[1]Royal Veterinary and Agricultural College, Horsholm, Denmark.

recognize that large areas which have benefited from great timber reserves in the past do not always possess the most favorable climatic conditions today for tree growth. Other parts of the world with higher temperatures, greater precipitation, and stronger light are perhaps preferable. Hereditary selection and tree breeding should perhaps be introduced in the less naturally favored regions. In the future there will be greater and greater requirements for wood and a realistic view of the possibilities of genetics in silviculture must be kept in mind.

It is not surprising that the problem was ignored in those parts of the world where nature seemed to furnish an inexhaustible supply of wood. It was not the pioneer, turning his cattle loose into a sea of grass, who took up the problem of grass improvement. It was resolved and solved in those countries where the greatest number of cattle of the best quality had to be fed in the smallest possible area. In the process it became apparent that other and more desirable varieties of grass could be advantageously cultivated on a large scale.

Even in areas very deficient in tree growth there seemed no incentive to practise plant breeding though improvements based on that science were giving better results in both agriculture and horticulture. It was natural to hesitate to limit forestry merely to the culture of cuttings and graftings, though these were being used with advantage to improve parks and gardens with trees of unusual beauty. Although astonishing results had been achieved by means of controlled pollination and selection in herbaceous plants, to attempt similar control in trees involved clambering about in the tops of tall trees and the prospect of obtaining as many generations as farmers and gardeners required to obtain seed-true types seemed hopeless. Forestry was, therefore, actually forced to find some new and better methods to replace the poor stands and poorly yielding species of trees.

In large sections of Europe beech and oak had been replaced by Norway spruce; about a hundred years ago the rapidly growing conifers from western North America began to dominate the scene in many European countries. With the use of exotic species of trees, especially those brought from a great distance, it was apparent that it was not always "the same thing" which was being cultivated even though, botanically, it was the desired species. Douglas fir, introduced into Europe about a hundred and thirty years ago, is an example. David Douglas collected seed near Fort Vancouver on the Columbia River and sent it to London. By a stroke of luck, this first import was a type well suited to the climate of the British Isles. Thirty to forty years later beautiful forests could be seen developing from these trees; and as by that time far better communications had been established with the northwest coast of North America, a large amount of seed was imported to Europe from many different places which were the natural habitat of this tree. Its cultivation in Europe spread to places with a vastly different climate from that of the British Isles. The result was unique confusion. Seed of this single species produced in some localities the most magnificent stands, in others, plantations of a luxuriant green type, which was immediately damaged or killed by frost, or the variety *glauca*, so slow-growing that it could not compete with our common spruce (*Picea Abies*). These and similar results helped awaken interest in silviculture and led the Forest Research Organization to set up a series of so-called "provenance-trials" which later proved to be of great value.

However, similar experiments on a large scale were not planned before the turn of the century. This delay now seems strange, for as early as 1820–40 Ph. A. de Vilmorin had conducted experiments which plainly demonstrated the existence of *races* of forest trees. Unfortunately, these experiments went unheeded by silvicul-

turists. About 1900, increasing knowledge of genetics acted as a stimulus to experiments which were further supported by Göte Turesson's work with ecotypes. The many experiments made hitherto have led us to see that the best way to avoid unpleasant surprises is to use seed from the same climate as that from which the desired form was imported.

Concurrent progeny tests have been made of single trees where certain characteristics were prominent—the columnar oak (*Quercus robur* var. *fastigiata*), the weeping beech (*Fagus silvatica* var. *pendula*), and the snake spruce (*Picea Abies* var. *virgata*). The results have been used to convince silviculturists of the value of genetics as applied to silviculture.

After a long series of experiments in the first twenty to thirty years of this century, complete certainty was reached as to an important genetic variation in forest trees. It became generally acknowledged that the geographical provenance of seed was of prime importance. Attempts were also made to avoid seed from poorly developed forest stands. The first steps towards improvement by breeding had been taken though, as yet, selection was made on the basis of free, non-controlled pollination.

More than one hundred years ago it was recognized that foresters could perhaps obtain better trees by raising and using hybrids (Klotzsch, 1854). This idea, however, was just as little heeded by foresters in general as were the hybrids of the American species of Juglans developed by Luther Burbank, 1877–8.

The hybrid larch of Scotland was the first tree to seriously hold the attention of foresters. It had been developed from the seed of some Japanese larches which had been pollinated by the earlier, widely planted European larch. Ever since the first detailed report in 1915, the tree had been the object of much attention. Here was something really new, something not actually and solely from nature's own rich supply of forms. Here was a combination of valuable characters derived from each of two good species which by crossing had produced progeny with pronounced heterosis.

To study the heredity of the branch types of Norway spruce, N. Sylvén, in 1909, conducted experiments in self-pollination. In these the progenies were compared with plants from free pollination. After this, artificial pollination was used in an early experiment at Placerville, California, to produce the hybrid *Pinus radiata* × *attenuata* (1928). A. Dengler, also using controlled pollination, made valuable experiments with *Pinus sylvestris*, on which he made a report in 1932. Special interest was awakened in the subject when A. J. Kolessnikoff, at the International Congress of Forest Experiment Organizations in Stockholm, 1929, presented results from successful self-pollination of several species and recommended "inbreeding a stage further."

Thus, over a period of several years, there appeared no small number of *strong appeals for controlled pollination in experimental work in silviculture*. Perhaps even more remarkable is the fact that the use of vegetative propagation was proposed. The Swedish scientist, Gunnar Andersson, made such a suggestion as early as 1906. He zealously argued that foresters should select good seed-trees in the finest stands, and that it would be beneficial to follow the example of gardeners and use grafting on a large scale. Unfortunately, his advice was not taken, and the same is true of a program for improving forest trees by breeding which the German, L. Fabricius, put forward in 1922. This scientist claimed that plantations for seed production could be established with vegetatively propagated material. In 1923, A. Oppermann, leader of the Danish Forest Experimental Research Station, published an article on

the larch. He mentions the possibility of grafting the most valuable trees to obtain more seed. Strangely enough neither of these two reports had any influence on experimental work in silviculture.

Americans were the first who were courageous enough to apply the adjective "tree breeding" to a forest research station. In 1925 the Eddy Tree Breeding Station was opened at Placerville, California. It was established by private initiative, but was later, under the name Institute of Forest Genetics, linked to the Forest Service. From the very first the genus *Pinus* was the primary object of study at the station. In the eastern part of the United States, at about this time, a task similarly restricted to the improvement of poplar was taken up. With the support of the Oxford Paper Co., A. B. Stout and E. J. Schreiner carried out many crossing experiments by which they succeeded in raising valuable new types. The results attracted attention and aroused hope for further improvement of forest trees. The work was later continued at the Northeastern Forest Experiment Station.

W. von Wettstein, in addition to studies of various tree species, demonstrated that cuttings from poplars can be made to flower and after controlled pollination to bear ripe seed. This was a long step forward. Now, to a greater degree, the work which Wettstein himself had advanced could be organized on sound lines.

The poplar seemed a particularly attractive subject for study. Because of the easy production of hybrids—a large number had appeared at random—and because the results could be used in vegetative propagation, the poplar seemed well adapted to improvement by breeding. It was with this very genus that Heimburger introduced forest-tree breeding into Canada. We were all enthusiastic when H. Nilsson-Ehle, in 1935, found the triploid aspen (*Populus tremula* f. *gigas*) in Sweden. With his unique and world-famous contribution to other fields of plant breeding as background, Nilsson-Ehle by his discovery and subsequent speedy breeding of tetraploid plants raised great expectations that valuable results would be derived from forest tree breeding. Through private initiative the Förening för Växtförädling av Skogsträd (Society for Breeding of Forest Trees) was founded. Nilsson-Ehle was the driving force in the early years and N. Sylvén became the first director of the society. On his own initiative Bertil Lindquist, about the same time, carried out active work in Sweden particularly concerning the utilization of the narrow-crowned Scots pine.

I do not intend to take time to give a complete historical account of tree breeding. If I have omitted anything I should have mentioned, may I be pardoned. My aim has been to give an impression of the development of breeding in silviculture up to about twenty years ago when forest-tree breeding began in many places to attract attention and interest. In Denmark I have been happy in being able to take part in this development. In 1924 I made my first controlled pollination experiment with *Abies*. Some of these plants I have today. Since 1930 controlled pollination has been used on a large scale, and in particular a series of rather successful pollinations in the early years with larch encouraged the continuation of the work. In the spring of 1934, I made the first graftings of ash with material from selected breeding trees. After that, vegetative propagation in conjunction with controlled pollination became the basis of the development of forest-tree breeding in Denmark. Broadly speaking, it has followed the lines which I mentioned in the same year in a paper on forest-tree breeding, from which the following is an extract: "I strongly urge, therefore, taking up vegetative propagation and, in conjunction with experiments in artificial pollination, the establishment of seed plantations for the supply of seed for practical use" (Royal Veterinary and Agricultural College, *Yearbook*, Copenhagen, 1934). That

is presumably the background for my presence here today as a senior worker in this field.

As already suggested some of the biologically most distinctive characteristics of trees are the advanced age at which the individual tree bears fruit, the inaccessibility of the flowers, growing high up in the tree, and the bulky structure of the mature individual which renders it practically immovable.

We are inclined to see these factors as difficulties in plant breeding. However, there can also be a great advantage in working with long-lived material. Furthermore, the individual tree often bears great quantities of seed, and, generally speaking, all of them can be propagated vegetatively.

In forest-tree breeding we will not choose between vegetative reproduction in clones and generative propagation to develop true-to-seed strains. We combine the two methods. Using both simplifies the task.

By grafts or cuttings from a selected tree we can obtain plants with the same inherent tendencies (genes) as in the uppermost flowering branchlets or in any other part of the tree. *Vegetative propagation* may be carried out in different ways and the material subjected to varying treatments, depending on the purposes for which the plants are to be used. Much time can be saved by investigating the inherent tendencies of the tree—and thereby its breeding value—by both the generative and the vegetative methods. Using both of these methods eliminates the significance of distance since the living, vegetative material can easily be shot down from high trees and sent over long distances. After appropriate insertion it may then be brought into early flowering, or used to test directly the qualities of the vegetative material.

In the large crowns of selected trees there will be such a quantity of twigs that we can at once begin experiments with a multiplicity of genetically identical plants. We can thus preserve the selected trees as living specimens in any desired number. Unlike other plants trees do not die after a year or two. This makes it easy to continue *controlled pollination* on a large scale. It is thus possible in a small experimental area to make simple and quick controlled pollinations between high grade trees which otherwise would be separated by great distances.

At the present stage in the development of silviculture it is advantageous to be able to increase the amount of forest seed. On fifteen-year-old graftings of the European larch we have harvested 1 kg. (200,000 seeds) per plant. A very few graftings yield far more than can be gathered from a large old tree—and the expense is much less. Furthermore, by grafting we are able to move the individual away from a spot where it would be liable to chance pollination. On isolated areas we can compare graftings from a selection of phenotypically promising individuals and build up "seed-gardens." Thus it is possible to achieve the objective of *producing seed by controlled pollination in whatever quantities are desired.*

When there is need for seed, or when it suits one's own convenience, the work of building up "seed-gardens" on the basis of promising phenotypes may begin. Or, more cautiously, one may first again sort out carefully the material to be used. The cost is so slight that both can be done. At all events the method gives us a guarantee that we can utilize a tested good material for production of seed in large quantities and it gives us courage to undertake improvement in plant breeding.

Controlled pollination is the simplest way to obtain knowledge of the value of trees for breeding purposes. Primarily it is important to be able to designate good genotypes. Here "tree shows" are useful. By this is meant clone testing, where in

practice grafting has been used on a large scale, with necessary care shown in the selection of stocks and scions. It is easy to produce enough material so that several clones can be placed under identical conditions, and single clones tested under different conditions.

This is our means of obtaining in a few years some approach to those qualities which have caused a tree in the course of a long period in the forest to develop into a specimen which is desirable from one point of view or another. Instead of waiting many years for an answer, we are helped by a rapid analysis of the past.

Although this does not provide the complete answer to the question of obtaining good breeding material—that is, plants for our "seed-gardens"—it goes a long way towards doing so. The question is not only one of an estimate of the genotype of the individual tree, but the possibility for estimating its separate tendencies.

We must remember that silviculture is still at a comparatively primitive stage in regard to the application of genetics. Therefore we should not overlook the value of "tree shows." The striking difference from clone to clone as it becomes apparent in a tree show has an almost overwhelming effect on a forester. To see the differences from clone to clone, it is not necessary to begin with phenotypically very different examples—even apparently similar specimens are often seen to differ greatly from each other after a closer comparison.

Tree shows exemplify not only differences in habit and growth vigor, but also differences in conditions of leaf-flushing, leaf-fall, flowering, and wood anatomy. Used in a pathological evaluation of individual specimens, they are invaluable. After we have collected our "patients" in order to present them all lined up in their "hospital beds," it is the moment to summon the specialists.

Long before observations can be utilized effectively in forest-tree breeding, they may have been useful in silviculture as indicators for seed collecting, plant nurseries, and treatment of present forest stands. As meteorological observations are also important in silviculture, and as we are often dependent solely on easily measurable factors such as precipitation and temperature, we might obtain additional information in a selection of "type trees" of various species whose clones could be used for registering the climate of different localities as "living meteorological stations." Another use for genetics in silviculture!

The use of clones, then, is very important in silviculture. We must, however, remember that they should not be used as the principal means of direct plant production. It should not be our aim to base timber production on the direct distribution of clones, as does the gardener when he plucks his roses or grows his fruit on clones—or as is done in raising poplars. Vegetative propagation has its primary function as a technical aid. This difference in viewpoint must be kept in mind. In the beginning it was difficult to comprehend. I remember that my paper on the subject at the International Silviculture Congress in 1936 caused a reporter to write that I would breed forest trees vegetatively and proposed exchange of material from the good genotypes. Probably the same difficulty explains why the earlier suggestions from Gunnar Andersson and L. Fabricius were not followed.

The *aim of forest-tree breeding*, or as I wish to emphasize here, the *application of genetics to silviculture* must be to establish the closest possible relations between silviculture, genetics, other forms of tree breeding, and botany. The last category should include botany in general, dendrology, plant pathology, and plant physiology. Such co-operation would enable silviculturists to profit from results already reached in "other fields," and, let us hope, tempt biologists to solve problems which may be applicable to silviculture.

With these long-lived trees, pollinated by the wind and well adapted to vegetative propagation, we have the ideal material for polycross and combining ability. Seed-gardens will have special significance for the production of seed for hybrids and thus for making use of heterosis in plant improvement in forestry. With our paucity of species here in the north, we will probably combine heterosis with inbreeding. Under other climatic conditions, with countless closely related species at hand, it would be interesting to attempt a more direct application.

And so we silviculturists call on you for help in establishing genetics in silviculture. We hope you will respond to our urgent request.

SYMPOSIUM

GENETICS IN ANIMAL BREEDING

1. H. NACHTSHEIM. Problems of Comparative Genetics in Mammals
2. ALAN ROBERTSON. Laboratory Breeding Experiments and Animal Improvement
3. C. STORMONT. On the Applications of Blood Groups in Animal Breeding
4. H. P. DONALD. Evidence from Twins on Variation in Growth and Production of Cattle
5. IVAR JOHANSSON. The Genetics of Milk Yield

PROBLEMS OF COMPARATIVE GENETICS IN MAMMALS

H. Nachtsheim[1]

IT IS DIFFICULT to report on comparative genetics—even of mammals only—in thirty minutes; to do it *in extenso* is impossible. I will, therefore, restrict myself to the discussion of just a few problems. May the first example, which is especially relevant, serve as an introduction, and at the same time bring to mind the importance of comparative genetics.

Thirty-one years ago at the V International Congress of Genetics in Berlin, Hermann J. Muller reported, for the first time, on his successful experiments to increase the mutation rate in *Drosophila* by X-rays. This discovery was not only the starting point for the development of a new field of research, radiation genetics, which was to be of great theoretical significance but it was also of practical importance and deserved special attention in general medicine.

In 1931, Paula Hertwig, in a lecture at the German Association for Genetics, reported on previous results in radiation genetics and then, with great emphasis, warned gynecologists of the serious danger to future generations if unnecessary use of X-rays were made in diagnosis and therapy. She especially condemned the direct radiation of reproductive glands in so-called temporary sterilization. In a resolution of the German Association for Genetics and the German Association for Eugenics, the members accepted her warning and threw down the gauntlet to the gynecologists. In the lively discussions between the disputing parties, the gynecologists always objected with the stereotyped phrase, "Man is no fly." The resolution of the geneticists rested exclusively—so the gynecologists said—on experiments with insects and plants, and before passing resolutions the geneticists had to show and to prove that man would react to the rays just as the fly did. The attitude of the geneticists would only endanger the further development of the indispensable and irreplaceable diagnostic and therapeutic application of X-rays to the abdomen.

Richard Goldschmidt, at that time chairman of the German Association for Genetics, answered that the point of view of the gynecologists would not change that of the geneticists. The attitude of the gynecologists was based mainly on the argument that experiments with plants and insects should not be valid for man, an argument incomprehensible today to everybody acquainted with the results of modern genetics.

However, Paula Hertwig made a concession to the gynecologists inasmuch as she noted that it would be desirable to confirm the results from experiments on *Drosophila* and other organisms by experiments with mammals in order to convince non-geneticists. Her own genetic experiments with radiation on mice accomplished valuable pioneer work. She found that the mouse reacts to X-rays in respect to its hereditary material basically in the same way as *Drosophila*, but the mouse is much more ray-sensitive—according to the latest American experiments about fifteen times as sensitive as the fly. This greater ray-sensitivity seems to be typical for all

[1]Max-Planck-Institut für Vergleichende Erbbiologie und Erbpathologie, Berlin-Dahlem, Germany.

mammals. The gynecologists had been right in saying "Man is no fly," even if they meant it in an entirely different sense. Or, would they now oppose us with the new slogan "Man is no mouse"?

Comparative Genetics within Species

First of all, a few facts about comparative genetics within the species must be borne in mind, if we want to compare more or less widely diversified types. It is almost commonplace today to say: identical phenotype does not mean identical genotype. In mammals, as in other animals, there are numerous cases of *mimic genes* or *genocopies*, as I like to call them in contrast to phenocopies.

I shall start with an example from my own experiments. I find three *short-hair* genes of the rabbit, producing three completely identical phenotypes, called French-rex, German short-hair, and Normandy short-hair (international symbols *R-1*, *R-2*, and *R-3*, respectively). All three genes show the same simple-recessive mode of inheritance, R-1 and R-2 belonging to the same linkage group III, with a crossover percentage of 17.2 ± 0.4. At first, type 2, the German short-hair, apparently had wavier hair than the other two types, but it could be shown that the stronger waving depends on a modifying gene, which in combination with each of the three short-hair genes in homozygotes intensifies the waving of the hair; however, in the genotype of normal hair, this modifying gene is ineffective. Likewise the combination of two short-hair genes succeeded in double recessive homozygotes, but the "double short-hair" differs from the simple short-hair only by a delay in the development of the hair coat after birth. In general, however, no summation of the effects of the mimic genes takes place in this case.

A further example of genocopies, taken from my own experiments, is *keratosis* in the rabbit, which shows itself in the phenotype as furless, that is to say, as a deficiency of woolly hair. The increased cornification of the skin makes the penetration of the fine woolly hair impossible; only the coarse guard-hair can penetrate, and in the most extreme cases these too appear in greatly diminished numbers. Again, three different genotypes are known: *furless-1*, *-2*, and *-3*. Between the three simple-recessive types, there exist small differences in the degree of cornification and accordingly in hairiness. Furless-1 is the most extreme type with strong cornification, and only relatively small amounts of guard-hair; furless-2 is less cornified and therefore hairier; furless-3 completely lacks woolly hair, but has quite an abundant coat of guard-hair. By comparing the three types, one is reminded of the effect of different alleles of a series, but crossing of the types with one another furnishes proof for the effectiveness of the three different genes. The influence of genetic background on the shape of the characters in keratosis could not be determined. Penetrance and expressivity in all homozygotes are complete, even if there is a certain width of variation of the character in all three types, so that the variation curves overlap one another in their extreme forms.

Even polymorphous syndromes with numerous symptoms can arise in the same way under the effects of various genes. A good example are the mutants *crinkled* and *Tabby* in mice. Figure 1 shows the pleiotropic effects of the crinkled gene, according to Falconer, Fraser, and King (1951). The most striking feature is the abnormal texture of the hair coat. It looks ungroomed, a condition traceable to a disturbance in the development of hair follicles during embryogenesis and in the period shortly after birth. Further symptoms of the phenotype crinkled are bald patch behind the ears, bald tail, kinked tip of the tail, reduced eyelid aperture, corneal ulceration, respiration disturbances, and modification of the agouti pattern.

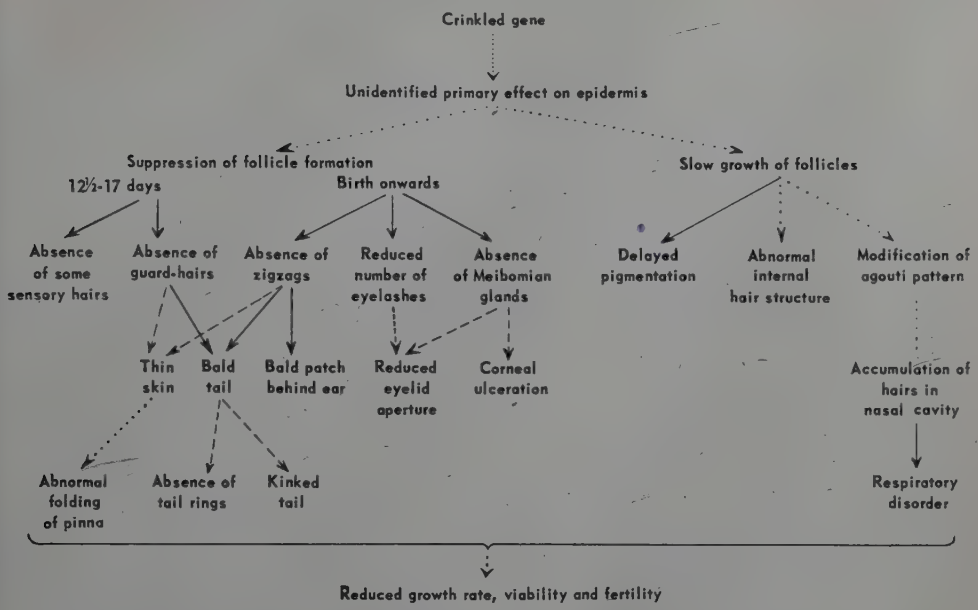


FIGURE 1. Pedigree of causes showing the origin of the pleiotropy of the crinkled gene. Not all the causes have been demonstrated with equal certainty. Those that we regard as proved beyond reasonable doubt are shown with a solid line: those that we regard as only probable with a broken line, and those that are no more than conjectural with a dotted line. (From Falconer, Fraser, and King, 1951.)

We could also label our chart, "Pleiotropic effects of the Tabby-gene in mice," as according to Falconer (1953) the symptomatology is just the same.

But there is one noticeable difference in the mode of inheritance. While crinkled shows a simple-recessive autosomal mode (linkage group XIV), Tabby is completely sex-linked, and is semidominantly inherited in the female (linkage group XX). The hemizygous Tabby males correspond to the homozygous females. In the heterozygous female the complex of features is expressed in a weaker manner. So the X-chromosome with the normal allele has a certain influence on the phenotype of the heterozygous female, whereas the presence of the Y-chromosome in the hemizygous male is without any effect on the Tabby phenotype.

In view of the far-reaching pleiotropy of the syndrome and the complete conformity of the phenotypes of both crinkled and Tabby on the one hand, and of the difference of localization and the mode of inheritance of both genes on the other, a translocation of the crinkled gene from the chromosome XIV to the differential segment of the X-chromosome and a change of the dominance relationship by a position effect were to be surmised. In this case, an interaction of both genes in F_1 would result. The crossing experiments, however, showed that there is no interaction between these two genes: the Tabby-crinkled individuals apparently do not differ from those which are Tabby or crinkled. Falconer, therefore, correctly came to the conclusion that the entire syndrome is the result of a developmental defect, at first visible in the inhibition of the hair-follicle development. The two genes would intervene in the same sequel of processes on different positions and these various changes would then result in the same end product.

One of the most predominant patterns of genocopies is exemplified in mice by *choreiformal motion disturbances*, or more briefly expressed by "dancing mice."

Besides the well-known Japanese dancing mouse, which, if Keeler and Fuji (1937) are correct, is the oldest observed mutation in mice, and in fact in all mammals, we know today more than a dozen different gene types, which are characterized by a more or less modified syndrome (Table I). There are essentially three main symptoms in dancing mice: (1) turning movements, (2) shaking of the head, and (3) deafness. But, on the whole, the picture of all the different types is extremely colorful and varied. Some particular types, although genetically differently determined, may completely conform in phenotype (as shaker-1 and shaker-2), but most types have their peculiarities. One or the other symptom may be missing (head movements, deafness); penetrance, expressivity, or the onset of the disease may be different. In some types the shaker syndrome is connected with symptoms of quite another kind, for example, in the short-tailed shaker, or in the shaker with syndactylism.

As Table I shows, the mode of inheritance in eleven of the fifteen chorea types is monomer-recessive; one is said to be dimer-recessive, three are semidominant. At least ten of the fifteen types are deaf. In general, the viability of the dancing mice is good, but merely by the addition of further pathological symptoms to the chorea syndrome mortality is high, as in the short-tailed shakers; or it leads to lethality in the first month of life, as in the syndactylous shakers.

The thorough investigations of Grüneberg and his co-workers, especially on the organs of hearing of the different types, have given us, in recent years, valuable information about the origin of the chorea syndrome, and its differential diagnosis. Grüneberg divides the choreatic types into two groups according to the findings on the labyrinth. In the animals of the first group, to which types 1–6 of Table I belong, the rough structure of the labyrinth is normal, but degenerative variations are present in its neuro-epithelium, and in the ganglion cells. These changes come into existence during the differentiation period of the labyrinth after birth, and they are usually of an advanced type. Most of these types react, from the clinical and pathological standpoint, in a very similar manner. All genes influence the cochlea and the vestibular apparatus. According to Grüneberg, certain differences between the existing types should be traced back not so much to the specific genes as to differences in the genetic background of the various stocks.

In the second group, types 8–14 of Table I, there are coarse anomalies in the form of the labyrinth, which begin in early embryonic development. After birth, however, they may lead to a small amount of further progressive degeneration. Among the various types of this group a large variation in malformation exists.

The genetic analysis of the chorea-types is today so far developed as to enable us to say: choreatic disturbances in the motion-reactions of the mouse may arise through mutative changes of numerous genes localized in different chromosomes, affecting different points of the development process, but leading in this effective chain of genes (Genwirkkette) to the same or a similar end-product, that is, to the many genocopies.

In mice and rabbits, as in other small mammals, it is relatively easy to demonstrate by cross-breeding experiments existing genetic differences in the same phenotype, that is, to demonstrate the existence of genocopies. It is more difficult in large mammals, and becomes impossible in humans, as this method of analysis is not workable. It cannot be doubted that many human diseases, such as epilepsy, chondrodystrophy, and rheumatism, are clinically and genetically collective terms, and that each one consists of a more or less large group of genocopies and perhaps also

TABLE I

CHOREATIC TYPES IN THE MOUSE

No.	International nomenclature: Name	International nomenclature: Symbol	Linkage group	Mode of inheritance	Behavior	Hearing	Viability (lethality, fertility)
1	Waltzer (French—valse)	v	X	Recessive	Dancing	Deaf	Good viability
2	Shaker-1	sh-1	I	Recessive	Wider circles, slight dancing	Deaf	Good viability
3	Shaker-2	sh-2	VII	Recessive	Wider circles, slight dancing	Deaf	Good viability
4	Jerker	je	XII	Recessive	Like waltzer, and shaker	Deaf	Females poor mothers
5	Pirouette	pi	III	Recessive	Small circles	Deaf	Good viability
6	Varitint-waddler	Va	XVI	Semidominant	Modified shaker syndrome	Deaf	Females poor mothers
7	Culbute		—	Recessive	Somersaults, circling	?	—
8	Shaker-short	st	—	Recessive	Circus movements	Deaf	High mortality, mostly sterile
9	Circler		—	Dimer-recessive	Circling, jumping, Somersaults	Non-deaf	Good viability
10	Kreisler	kr	V	Recessive	Circling, shaking of the head	Deaf	High mortality, only a few become mature
11	Fidget	fi	V	Recessive	Modified shaker syndrome	Non-deaf	Males fertile, females seldom, poor mothers
12	Dreher	dr	—	Recessive	Shaking of the head, dancing	Deaf	Viability good, but females mostly sterile or poor mothers
13	Twirler	Tw	XV	Semidominant		Non-deaf	
14	Zigzag	Zg	—	Semidominant		Non-deaf	
15	Shaker with syndactylism	sy	—	Recessive	Rolling around the longitudinal axis	Deaf	Lethal in the first month of life

phenocopies. But, even clinically and genetically well-defined diseases may break up in groups of genocopies if a suitable method of analysis is found.

In human genetics the best example is *hemophilia*. Up to a few years ago, hemophilia was the classical example of sex-linked recessive heredity in man. When, however, the bloods of various families of bleeders were compared a surprising fact was discovered: namely, that the blood from some bleeder families may correct the defect in the blood-coagulation process of other bleeder families. This discovery clearly established that different families may be suffering from different hereditary defects of the blood-coagulation process, obviously originating from different mutations. This discovery established the first genocopy to the so-called classical hemophilia or hemophilia A, the genocopy referred to today as hemophilia B. In English reports these two hemophilias are also designated according to the nature of their defect: AHF = antihemophilic factor deficiency; and PTC = plasmathromboplastin component deficiency. Hemophilia A and B are both sex-linked and recessively inherited. For the time being, we cannot decide whether we are dealing with different coupled genes in the X-chromosome or with different effects of one compound gene on the same process, that is, on the process of blood-coagulation. The combined occurrence of AHF and PTC deficiencies in three cases already speaks well for the explanation of Vogel that in both these disturbances there are different mutations of a compound gene.

The discovery by Koller and co-workers of hemophilia B in 1950 was an important step forward in blood-coagulation research in man. Today we recognize a large number of factors which are effective in blood coagulation and which can be changed by mutations. All these changes result in one and the same clinical picture for hemophilia. Autosomal localized genes may participate too, but in these autosomal genes mutation possibly occurs less often. The blood-coagulation process is the best example of an effective gene chain in man. And hemophilia illustrates the origin of numerous genocopies. But such an elegant genetic analysis without cross-breeding experiments is certainly exceptional in human genetics.

Besides genocopies, phenocopies may also be suitable for comparative observation within the species, especially since we know that, in the spontaneously originating phenocopies, at least, genetic background may also play an essential role.

Comparative Genetics between Species

Animal breeders have known for a long time that in various domesticated animals many physical traits appear in a similar way during the period of domestication: albinism, leuzism, melanism, angorism, short-hair, hairlessness, keratosis, certain spotting types, giantism, dwarfism, etc. But simply stating these facts is not comparative genetics. We have to find out by morphological, physiological, embryological and phenogenetic investigations if homologous genes and eventually allele series are actually involved; how far the parallelism of the various types goes; if eventual differences could be shown to be type-specific; if in sufficiently analyzed types the compared traits belong to the same linkage-group, etc. The well analyzed *albino series*, shows where we stand today.

The albino series until the present has been the largest allele series which we know to exist in mammals. In Table II, I have summarized this series for the more important mammals, rodents and carnivores, used for experimental genetic investigation. The alleles lead from full pigmentation, step by step, to total loss of pigment

TABLE II

ALBINO SERIES IN MAMMALS

Mutation steps	Characters in the rabbit	*Oryctolagus cuniculus*	*Cavia porcellus*	*Rattus norvegicus*	*Mus musculus*	*Microtus arvalis*	*Peromyscus maniculatus*	*Cricetus cricetus*	*Mesocricetus auratus*	*Chinchilla lanigera*	*Chinchilla brevicaudata*	*Sciurus vulgaris*	*Myocastor coypus*	*Felis catus domesticus*	*Canis familiaris*
1	Full color	C	C	C	C	C	C	C	C			C	C	C	C
2	Intense (dark) chinchilla	c^d	c^k		c^i	c^i				C^i	C^i				
3	Chinchilla	c^{ch}			c^{ch}	c^{ch}								c^{ch}	c^{ch}
4*	Light chinchilla	c^l	c^d											c^b	
5*	(Extreme dilution)		c^r	c^r	c^e										c^e
6*	Himalayan	c^h	c^h		†				c^h	c^h	c^h	†		c^s	
7	Albino	c		c	c	c	c	c					c		c

*Types 4–6 are bred in general without the agouti gene.
†Found as single specimens in wild populations.

production: that is from wild-type to the so-called chinchilla types without the yellow pigment and acromelanism, or in other words from the incomplete albino to the full albino. The most dominant allele *C* (color) is the indispensable basic factor for full pigmentation, whereas the most recessive allele *c* leads to complete loss of pigmentation. The more thoroughly a species is examined, the greater the number of the ascertained alleles, and it should not prove difficult to increase this number further. However, the greater the number of steps from the full-pigmented animal to the full albino, the less the difference between the respective steps, that is to say, the more diminished is the difference between the successive phenotypes. Type 6, Himalayan rabbit, could be broken into three hereditary types, of which one new type would be put between 5 and 6. The animals show a very light pigmentation as well as acromelanism. Where Himalayan rabbits are normally white, here a sooty- or smoky-looking tinge exists in the juvenile hair but is lost later on. The other new type is nearer to the full albino and is put between types 6 and 7 of the table. The acromelanism is confined in general to ears and tail, the last retreating pigment centers. The rest of the extremities remain more or less pigment-free. This is the type we find as a rule in guinea pigs, in golden hamsters, and in the two South American chinchilla species. Full albinos in these rodents and in the cat are at present still unknown, as far as I know. The mutation has not yet reached the last step.

One word more about the albino series of *Chinchilla lanigera* and *Chinchilla brevicaudata*. These two South American wild rodents have given the name to the chinchilla rabbit. The French rabbit breeder, who raised this new rabbit breed before the First World War, chose the name because of its phenotypical similarity with the wild rodents of South America. He could not know that he had hit the mark genotypically as well. According to an unpublished investigation of Wolfgang Zimmermann in Mariensee, the hair coat of the two chinchilla types corresponds completely to that of the chinchilla rabbit, not only in phenotype, but also genetically, since the corresponding gene is also in the chinchillas an allele of the albino series. An allele, which originated by mutation in rabbits and other domestic animals

during the process of domestication, has already become a species and a genus character in the wild animal. Other rodents demonstrate similar examples of this kind, as we will see later.

Whereas the allele of the wild-type *C* reacts completely dominant towards all other alleles, and the last allele of the series, *c*, is totally recessive, the middle alleles, those of steps 4 to 6, show an intermediate reaction. Moreover, types 4 to 6 are more or less strongly modified by temperature, as we know from the brilliant and frequently confirmed experiments of Walther Schultz with acromelanistic Himalayan rabbits. The animals of step 6 are all born completely pigment-free and develop the pigment in the first weeks of life only in the extremities of the body or, as in the Siamese cat, predominantly there. It is possible, however, to produce pigment in all the remaining places of the skin and the hair coat which are normally white if one lets the shaved hair grow in the cold. On the other hand, pigment development is prevented in the extremities of the body by an experimental increase of body temperature.

Even in the "red" eye of these part-albinos, pigment production is obtained by growing corresponding tissue undercooled *in vitro*. In current experiments, the ophthalmologist Kleberger in Berlin is establishing in the Himalayan colored golden hamster the same reaction as in Himalayan rabbits, guinea pigs, etc. On the other hand, in no full albino was there ever produced even a trace of pigment in hair, skin, or eye.

Noteworthy in this respect is the reaction observed in rabbits which I produced, denoted as "synthetic albinos." They have been bred from a cross between the Himalayan rabbit with its allele for Himalayan coloring and the Vienna white rabbit with its gene for leuzism. The allele of the *C*-series in the double recessives suppresses, so to speak, the light blue eyes of the Vienna white and the Himalayan pattern is suppressed correspondingly by the gene for leuzism. In this way, we get a phenotype, which does not differ from that of the genuine full albino. In this phenotype it is not possible to produce pigment in skin and hair by means of cold. However, in those layers of the iris in which the Vienna white rabbit normally has pigment (back of iris, retina), and only in these, pigment can be produced by undercooling *in vitro* (Schultz).

My co-worker, U. Ehling, recently made an unexpected discovery in the albino series of the rabbit. In his experiments aimed at producing radio cataracts by X-rays for comparison with inherited cataracts of the rabbit, there were by chance a few albinos. They proved to be more resistant to rays than the pigmented animals. The experiments were repeated on a wider basis on animals with the various steps of the albino series. It could be shown that the ray-sensitivity of steps 1 to 3 does not differ, whereas the ray-sensitivity of the lower steps is reduced parallel to the reduction of pigmentation. This fact may be briefly stated without jumping to conclusions. Further experiments are necessary.

A character should be mentioned here which has been observed for a long time, in connection with a definite allele (c^i) of the albino series, but only with that of the rabbit, which somehow differs from the usual effect of the *C*-gene. I am thinking of the character of marbled eye, which is in general, if not always, a typical trait of the intense chinchilla. The dark chinchilla types have dark brown eyes, the lighter chinchillas have brown eyes. In marbled eyes the pigment is not equally distributed in the forward iris layer, but instead is arranged in the form of small and slightly larger irregular points and spots. Where pigment accumulations are lacking, the

forward iris layer is light blue. Depending on the number, size, and density of the pigment spots, the iris is lighter or darker marbled on a blue background. The iris can become darker as the animal grows older on account of increased pigment production, so that the dark marbled iris color finally appears almost uniformly brown, while in the other extreme the light marbled eye is almost blue.

It was assumed that the marbled-eye character depends upon the effect of an independent gene closely linked with the allele c^i. P. B. Sawin in the United States and I in Germany searched in vain for a long time to establish this. Finally, in 1941, I succeeded in separating the two genes and the combination with another allele of the albino series by breeding marder rabbits (light chinchillas without the agouti gene) with marbled eyes. Because of the war, breeding experiments were interrupted. But still the experiments performed permit the following working hypothesis to be advanced: the marbled gene is closely linked with the gene of the albino series; yet the marble gene is kryptomere towards the extreme alleles of the albino series, that is, the allele of the wild type leads to a fully pigmented eye even if the marble gene is present. The marble gene is of course as little effective in the full albino as are other pigment factors, while in combination with the chinchilla alleles the marbled eye becomes evident, even with one single dose of the marble gene.

Brief mention should be made of two further allele series which have to do with pigment development in mammals. The one series is especially interesting since it is present in a similar way in rodents and in carnivores. However, in the pattern development of certain alleles it shows characteristic differences between these two orders. The other series is remarkable because certain alleles in the homozygous condition produce distinct pathological phenomena with a lethal effect in rodents and carnivores.

The extension series *E* controls the production of the black pigment. Five alleles of this series are known to exist in rabbits. The effects of the three which are most important for breed production will be mentioned here. *E*, the allele of the wild type, in combination with certain other pigment genes controls the total blackness in rabbits. The most recessive allele of the series, *e*, reduces the black pigment down to a small residual amount; the animal is yellow-colored, except for the extremities of the body which have a more or less dark tinge. Between these two alleles *E* and *e* we have the allele e^j which produces the so-called Japanese color, a varitint spotting which distributes itself irregularly over the entire body, and is made up of smaller or larger black and yellow areas. The Japanese color has become a species character in the wild rabbit of Sumatra, *Nesolagus netscheri*. In the European hamster, *Cricetus cricetus*, the varitint spotting corresponds to the Japanese color and has become a species character of the wild type. Fairly frequently both the other types of the *E* series, pure black and pure yellow, appear as mutants in wild populations of the hamster.

The three *E* alleles are known to exist in dogs as well. *E* and *e* affect dogs in the same manner as they do rabbits, hamsters, and other rodents, the phenotypes being black or yellow (for instance, Boxers and Great Danes). Also, e^j yields a mosaic type, black and yellow, but it clearly differs in dogs from the Japanese pattern of the rabbit or the varitint spotting of the hamster. In dogs a pattern which is more striped than spotted is displayed, with black stripes on a yellow background. We define this striped type as brindle (*gestromt*). It is possible that the same allele has different effects, or perhaps different alleles of the same series are involved, one of which has been known up to now only in rodents, the other only in carnivores. Supporting the

latter alternative is the fact that a wild relative of the dog, the African hyena-dog, *Lycaon pictus*, shows as a species character the varitint spotting of the Japanese rabbit and the hamster.

There are two typically different types of *black-white spotting* in rodents and carnivores. One is denoted in rabbits as Dutch spotting, a recessive type, showing a wide band color, which in extremely white-spotted individuals influences also the color of the iris. The other type denoted in rabbits as English spotting is dominant or, more correctly, semidominant, and is more of an irregular spotting form; even in extreme white spotting the eye color is not affected. The degree of spotting will be more or less influenced in both types by modifying genes as well as by the main genes. In rabbits and mice the main genes for recessive and dominant spotting are linked. In rabbits the linkage is very close (less than 1 per cent crossing-over); in the mouse just the opposite is true (over 45 per cent crossing-over). An attempt has been made to explain this difference by assuming an inversion in the respective chromosomes of one or the other species, but this is, in my opinion, very speculative.

However, for the moment we are interested mostly in the dominant spotting on account of the more or less far-reaching lethal effect of the gene in the homozygotes. The most detailed experiments have been carried out in mice, in which the most dominant gene carries the symbol W (*white spotting*), and a second allele the symbol W^v (*white spotting viable*). While the heterozygotes are completely viable, the homozygotes—they are dark-eyed and white-spotted—suffer from macrocytic anemia from which the W/W mice die within the first three days of life; repeated intraperitoneal transfusions of normal blood prolong the life span up to twenty-seven days (maximum). The allele W^v has the same effect on the hair coat, but the lethal effect on the homozygotes W^v/W^v is diminished; they are suffering from a less severe anemia, are viable, and are able to reach sexual maturity. The combination of both alleles (W/W^v) has a middle position. Most mice with this combination die within the first six weeks of life, but occasionally an animal may reach sexual maturity.

In the field mouse, *Microtus arvalis*, the same dominant spotting mutation has been observed recently. The mutations of house and field mouse not only correspond to each other completely in phenotype and mode of inheritance, but also in the field mouse the M/M homozygotes suffer from macrocytic anemia, and die shortly after birth.

The homozygous English-spotted rabbits are equally dark-eyed and white-spotted with a diminished viability in comparison with the heterozygotes; usually they are not even raised by the breeders. Unfortunately, detailed blood examinations are lacking. Still the idea is forced upon us that a smaller degree of anemia (as in the W^v/W^v mice) might be the origin of their weakness.

We have known for a long time that in the harlequin-colored Great Dane whose spotting corresponds to that of the English-spotted rabbit, the homozygotes are also more or less weakened in their constitution. Blindness and deafness are not rare. Unfortunately nothing is known about the blood picture of these homozygous harlequin-colored Great Danes, which, like the homozygous English-spotted rabbits, are not bred in general.

And now just a glance at man and mammal. In comparing the genetics of the most highly developed mammal with that of the other mammals we are at the very beginning. That is to say, we know that many of the characters of domesticated animals are found in humans, such as albinism, baldness, spotting, dwarfism, and giantism. Also many pathological hereditary characters are common to both, but

TABLE III

HEREDITARY DISEASES IN MAN AND MAMMALS

Organ system Disease or anomaly	Man	Rabbit	Guinea pig	Rat	Mouse	*Peromyscus*	Cat	Dog	Pig	Cattle	Horse
Dermal system											
Albinism (albino series)	×	×	×	×	×	×	×	×			
Elephantiasis	×								×		
Hydroa aestivale	×										×
Hypotrichosis, atrichia	×	×		×	×	×	×	×	×	×	
Hypotrichosis/anhidrosis/anodontia	×										×
Ichthyosis	×				×					×	×
Keratosis	×	×									
Nervous system											
Ataxia	×	×			×	×					
Dystrophia muscularis	×				×						
Epilepsy	×	×			×	×					
Hydrocephalus	×	×		×	×						
Spina bifida	×	×			×						
Syringomyelia	×	×									
Sense organs											
Anophthalmus/microphthalmus	×	×	×	×	×			×	×		
Cataract	×	×		×	×			×		×	×
Coloboma	×	×		×	×						
Deafness	×		×		×		×	×			
Hydrophthalmus	×	×									
Retinitis pigmentosa	×			×	×			×			
Skeletal system											
Brachydactyly	×	×			×						
Calvé-Perthes' disease	×							×			
Chondrodystrophy	×	×						×		×	
Harelip, cleft palate	×				×			×	×		
Oligodactyly	×				×						
Osteopetrosis	×	×									
Polydactyly	×		×		×		×	×	×	×	×
Syndactyly	×				×				×		
Split-hand	×						×				
Tibial aplasia	×				×						
Circulatory system											
Hemolytic jaundice	×			×							
Hemophilia	×							×			
Hydrops fetalis	×	×			×						
Spherocytosis	×					×					
Pelger's anomaly	×	×									
Urogenital system											
Kidney anomalies	×			×	×						
Digestive system											
Absence of second incisors	×	×									
Metabolism and endocrine organs											
Adiposity, obesity	×				×						
Diabetes	×				×						
Dwarfism	×	×	×	×	×				×	×	×
Porphyria	×								×	×	

for very few have comparative genetic analyses been carried out. I would like to mention my own experiments on the Pelger anomaly in rabbits and man as an example. As for the rest I have a list (Table III) of hereditary abnormalities and diseases which are known in man and mammals, but this list cannot be regarded as complete.

I now return to the remarks I made at the beginning of this lecture. In 1931 the German gynecologists rejected the results of radiation genetics, declaring "man is no fly." They were wrong since man is subject to the general rules of heredity like any other living being. On the other hand, I must admit that for special questions, as for example those of radiation-sensitivity, it is somehow unsatisfactory to extrapolate from the fly to man. This is a stop-gap only. Man is a mammal and in so far as we cannot gain the necessary information from the study of man himself, a substitute has to be made by experiments with other mammals. Man is certainly the most highly developed, most differentiated, most complicated, and—in the atomic age—the most dangerous mammal for all living beings. We should intensify the study of his genetics with all our might. "The proper study of Mankind is Man."

LABORATORY BREEDING EXPERIMENTS AND ANIMAL IMPROVEMENT

Alan Robertson[1]

IN RECENT YEARS there have been many studies of quantitative inheritance in laboratory animals. We have now at our disposal a considerable body of theory of quantitative inheritance, which in its most convincing aspect provides a description and integration in statistical terms of diverse phenomena in static random-breeding populations. The statistical parameters are a consequence of the segregation of many alleles at many loci, but only rarely can we penetrate to the level of the individual genes themselves. Ordinarily, we have to content ourselves with coherence at the statistical level. The theory provides a rough guide to the reaction of the population to changes in the mating system, such as selection, but these predictions have no certain long-term value. Essentially, this is because the theory does not give us any good method of predicting how the parameters themselves will change. The theory has two main imperfections, even at the static level. It can deal with linkage only in rather imprecise terms (in that it can predict the nature rather than the exact magnitude of the effects) and it must assume, as far as concerns the genes affecting the characters we may be interested in, that there is no natural selection and that zygotes appear in Hardy-Weinberg proportions. This is a rather disturbing omission—we have to assume in the majority of our genetic analyses that the forces which shaped the genetic variation in the past are not now operating.

I should like to suggest that experimental work on the quantitative genetics of laboratory animals is relevant to animal improvement at two levels. First, I would put the examination of the validity of existing theory and the provision of evidence that would lead to its extension in the directions in which it is at present inadequate. And secondly, at a rather more fundamental level, the provision of evidence that may allow us to discuss over a wide range of organisms the relationship of the type of genetic variation to the kind of character that we are dealing with. The latter obviously leads us very directly to questions that are of great interest to the student of evolution.

Apart from the obvious advantages of time and cost, the use of small laboratory animals offers us great possibilities for detailed genetic analysis. If we find that theory is inadequate in a certain situation, we can make use of "trick" stocks to analyse the problem in much greater detail than we could in, say, pigs or cattle; and, perhaps, in so doing we can work out methods of diagnosing the same problem if it does occur in these animals. But I think we have to be extremely careful in extrapolating our results to larger animals. The results we may find in the laboratory with a particular character in a particular animal are a reflection of the genetic situation there, which will have been shaped by the forces of natural selection in that animal. I feel that at the moment extrapolation at the level of characters is extremely difficult. But the situation in a population can be described statistically and an extrapolation at this statistical level is much more meaningful. It will be clear that I do not believe that an operational and pragmatic use of laboratory

[1]Institute of Animal Genetics, Edinburgh, Scotland.

animals in, say, comparing different selection programmes is of great value unless it is linked with use of genetic and statistical methods for a full analysis of exactly what is going on.

The Adequacy of Our Present Theory

In *Drosophila* the main body of work has been done on bristle counts, body size, and egg production. In spite of its many advantages as an experimental animal, *Drosophila* has the disadvantage of being rather lacking in easily measurable characters which show variability. This undoubtedly accounts for the popularity of bristle characters. The other main disadvantage is that *Drosophila melanogaster*, the species most used because of its short generation interval, has only three long chromosomes and no crossing-over in the male. We may for this reason expect to find that linkage plays a greater role here than in other organisms though it should not be forgotten that the hen has perhaps no more than six effective chromosomes. The effect of linkage was clearly shown by Mather and Harrison (1949) in their classical work on selection for bristles from a cross between two inbred lines.

The only work specifically carried out as a detailed check on theory was done by Clayton, Morris, and myself (1957), again using bristles as the character and starting from a large random-breeding population. Observations made on the genetic variation in the base population were consistent with each other and, correspondingly, the predictions of short-term response to selection by different methods were on the average fairly accurate, though there were considerable differences between replicate lines. On the other hand, the genetic parameters and the response altered markedly with only two or three generations of down selection and the further selection proceeded, the less adequate became the static description of the population in the prediction of selection gains. In so far as explanations could be given for this inadequacy (and such analyses are very labour-consuming) they seemed to lie in the continued selection of heterozygotes for lethal recessives. In general, long-continued selection resulted in populations still retaining genetic variability though response had ceased.

The results of continued selection for body size by Forbes Robertson and Reeve (1952) were very similar, though they showed conclusively that the stable situation so reached in "up" lines was quite different from that in "down" lines in that the former could be rapidly selected back to their starting point whilst the latter changed little on back-selection. Many of these results exposed the inadequacy of our ideas on the symmetry of response to short-term selection. On existing theory, in which the immediate changes in the population mean produced by selection are due to small changes in gene frequencies, the response to equal selection intensity in the two directions should be symmetrical, a reflection of equal changes in gene frequency. There is little doubt that populations that have reached semi-stability in the face of long-continued selection do not often show symmetry—the effective heritability backwards is much greater than it is forwards. A possible explanation of this phenomenon would lie in the segregation of major genes (showing overdominance or lethality in the direction of selection) in which the gene frequency changes might not be small, or in the continued opposition of natural selection to artificial selection, as suggested by Lerner and Dempster (1951) for shank length in poultry. But there remain cases of asymmetry, such as those discovered by Bateman (1958) during studies on lactation performance in mice, in which an undoubted immediate asymmetry exists which has so far defied explanation, and others, such as the selection for bristles referred to above, in which the rate of

response changes surprisingly rapidly in one direction of selection for a character which presumably has not been under selection in natural populations.

The mouse occupies an intermediate position as a laboratory animal. It is more expensive per unit than *Drosophila* and laeks the special stocks which make genetic analysis so much easier. On the other hand, it has a great many chromosomes and, as a mammal, may be claimed to be physiologically somewhat closer to a pig or a cow than is a drosophila. We may be in a position to extrapolate, again with considerable caution, at a physiological rather than a statistical level. The majority of the work on mice carried out with a theoretical framework in mind has been done by Falconer and his group (1955). The general picture has been rather similar to that from the work with *Drosophila*—response for periods of from ten to twenty generations followed by stability but with considerable genetic variation still remaining. One particular aspect of work with mice, which has stimulated new lines of thought, has been the necessity for taking into account the effect of maternal environment on the development of the young. This problem has, of course, presented itself in work with pigs, but is more amenable to analysis in the mouse because of the comparative simplicity of egg transplantation. The mouse also lends itself well to the study of selection under different environments. Until comparatively recently, control of the environment in drosophila cultures had not been sufficiently developed for useful work in this direction, in addition to which the developing drosophila modifies its environment as it goes. The experiments by Falconer on selection for body size in mice on different planes of nutrition has been an excellent example of work in which extrapolation at a physiological level is meaningful.

The general picture that has emerged from all these experiments is one of reasonable agreement between observation and prediction for a few generations of selection for characters not previously exposed to selection, with perhaps greater agreement if the changes in the genetic parameters are allowed for. But, after long periods of intense selection, the value of the predictions falls off and we are left with some unfixable genetic variation. Presumably we would reach this situation rather sooner if we started with a character which has been under natural selection, such as egg laying. This is the problem of the poultry breeder with strains of birds that have reached a "plateau" after many generations of selection for egg production. How can we best utilize this unfixable, presumably non-additive, genetic variation? This aspect of selection, the utilization of heterosis, has not been adequately worked on in the laboratory and, unfortunately, most of the work on egg production has been pragmatic rather than analytical in its aims. We still require a thorough genetic analysis of some such character in a population in genetic equilibrium and of its reaction to selection of different kinds in both directions. Perhaps the most valuable work on such characters in this context has been the extensive studies of wild populations of *Drosophila* by Dobzhansky and his school, which have revealed the complexity of the situation when we deal with characters closely connected with fitness. The detailed work necessary for adequate analyses of such situations is laborious and unfortunately too few of them have been done.

Comparatively little has been done on the problem of genetic correlations between different characters and the related problem of the correlated response of characters other than those directly selected for. What studies there are would suggest a similar picture to that involving direct response—reasonable agreement in the early generations and rather less later on. However, the work of Forbes Robertson and Reeve (1952) on wing length and thorax length in *Drosophila*, in which the genetic correlation is high ($\sim$0.7), showed good agreement between predicted and observed

response in the second character over some twenty-five generations. Here I should mention the work of Falconer (1952) on selection in mice on different planes of nutrition, which has been valuable in leading to a new theoretical framework for the consideration of problems of genotype-environment interaction.

The greatest dearth of both theory and critical evidence is in the field of genetic inter-relations between metric characters on the one hand and fitness and its components on the other. It is not exactly original to say that, if we alter the array of gene frequencies in a population, the fitness will decline (and consequently its components). We may expect the decline of fitness in response to random changes of gene frequency, that is, inbreeding, to be linear in the early stages though at later stages Forbes Robertson and Reeve (1955) have put forward some evidence to suggest that the effect of any segregation is greater at higher levels of homozygosis and that we might, therefore, expect the decline to accelerate. In a programme of selection for a metric character without deliberate inbreeding, we have very few clues to what we may expect although, taking a rather simple view of the situation, the fitness should decline as the square of the response to selection. However, in most selection experiments, the degree of inbreeding has not been kept low and the two effects have been confounded. One of my colleagues has been working on this problem in *Drosophila* and his results would suggest that the population concerned is more affected by changes in gene frequencies by inbreeding than by selection. To be more precise, in a population in which ten flies out of fifty of each sex are selected for abdominal bristle count (leading to an expected change of one genetic standard deviation each generation) the decline in fitness of 40 per cent at the fifth generation of this intense selection and mild inbreeding could be attributed roughly equally to the effects of inbreeding and of selection.

Finally, we have little theory and almost no evidence, as to the extent to which the intensity and method of selection influence the selection limit eventually reached. If the population size during selection is large, then simple theory would suggest that the limits are dependent on the number of genes concerned (implying, for a given amount of variation, the magnitude of the individual effects concerned and the array of gene frequencies). Here we have little evidence, but the fact that in many different selection experiments the limits have been reached after some twenty or so generations would suggest that in these experiments the effects of the most important genes are of the order of one-quarter to one-half of a phenotypic standard deviation. In practice, we have imposed on this simple picture the complications of inbreeding and linkage. Separately, these two can be dealt with theoretically, but together they make up a problem of such complexity that only Monte Carlo methods and a digital computer can provide the answer. At the factual level, we have almost no experiments in which selection limits have been investigated using different population sizes and intensities of selection.

The Spectrum of Genetic Variation

Now let me turn to the second aspect of work with laboratory animals—the information it has given us as to how the different kinds of characters behave—in other words, the nature of the genetic variation. In many characters, we find in random-breeding populations an extraordinary amount of variation—expressed in terms of the limits to selection in the two directions. Why some characters should be variable and others should not is not very clear. Indeed, it is not entirely clear what scale I should use to say character A shows more variation than character B.

As regards the nature of the genetic variation, certain generalizations would seem to be possible, and some aspects of evolutionary theory would suggest that they are reasonable. Different characters seem to fit in some kind of order. In *Drosophila*, we can fit our bristle characters at one end and characters closely connected to fitness, such as egg laying, at the other. In a population, which has not been subject to artificial selection, abdominal and sternopleural bristles show no change in mean count on inbreeding and the additive genetic variance makes up a high proportion of the total genetic variance. Experiments in which the effects of "high" and "low" chromosomes have been studied in different genetic backgrounds show little or no interaction between non-allelic genes. Body size shows some but not a great deal of depression on inbreeding, a fair proportion of the total genetic variance is additive, and there is evidence of interaction between genes on different chromosomes. In egg production, on the other hand, inbreeding has a considerable effect, only a small proportion of the total genetic variance is additive, and we have evidence from the F_2 of crosses between different populations that non-allelic interactions play an important part, that "the gene-pool is integrated." I think that we must conclude from the evidence on bristle characters that at the individual gene level there must be additive action (or something very close to it) in the sense that the heterozygote must be nearly intermediate between the two homozygotes. At the other extreme, in egg production, we see the effects of the dominance-recessive relation—with perhaps the effects of overdominance thrown in—in the inbreeding depression and the large amounts of non-additive genetic variation. I have suggested elsewhere that this pattern of behaviour is probably related to the causal connection of the character with fitness. At the gene level, we have additive action in mere existence—at the level of fitness, when the effects of the segregation have been modified by the evolution of dominance and by canalization, we have dominance. We might expect to find a gradual increase in dominance as we proceed along the causal path from gene to fitness and in any observable character the genetic variation will be shaped by the causal relation of the character with fitness. I have given illustrations from *Drosophila* because there we have the greatest amount of evidence. But characters in the other laboratory animals and in domestic animals fit fairly well into the pattern. We must remember, however, that domestic animals have evolved in an environment vastly different from modern farm conditions and that exact parallels need not apply.

Perhaps we can fit into this pattern the greater variability that has been found in heterozygotes compared to homozygotes. If the lack of vigour of the homozygote is a consequence of greater sensitivity to unaccustomed environments, we might expect the effect of homozygosis on variability to be least at the bristle end of the scale and greatest at the fitness end. If we further express this pattern in terms of the coefficient of variation, the effect will be magnified by the inbreeding depression. But there is little doubt that in this and other aspects of the subject, we still need considerably more straightforward observation of how different characters respond to selection and inbreeding.

But why do we find all this genetic variation in our populations? This question requires answers at two different levels—why are these particular genes segregating and why are they affecting these particular characters? These are obviously interrelated problems, but they differ vastly in complexity. We at least know the terms in which to answer the first one—it is merely a question of doing the right experiments. I rather doubt whether we as yet know how to ask the second question in such a way as to get a meaningful answer.

At the gene level, we have to consider in a closed population the forces of mutation and natural selection in relation to the probable effective breeding size of the population. Natural selection may act as a preserver of genetic variation if the heterozygotes are favoured, or as a remover of it as in selection against homozygotes for deleterious recessives. In relation to quantitative variation, we have, however, very little idea of the magnitude of these forces. As regards the rate of origin by spontaneous mutation, what numerical evidence is available refers only to bristle variation in *Drosophila*. Here the new variation is detectable and that arising each generation is apparently of the order of 1/1,000 of that found in wild populations: that is, if mutation was the only force operating it would take around a thousand generations for a homozygous inbred line to acquire the level of variability of wild populations. This may appear low in the scale of laboratory experiments but may on an evolutionary scale be sufficiently high that we have to take it into account in discussing the balance. That such a small effect can be of importance indicates the experimental difficulty in attacking the problem. Here we have a situation in which we can only hope to get an answer in an organism in which the generation interval is short. I might mention that even irradiation is apparently a very slow way of producing new variation in bristle characters—our best estimate of the total dose to produce variation equal to that in wild populations is several hundred thousand roentgens.

At least three different approaches to the investigation of the strength of the forces of natural selection would seem to be possible: (*a*) the effect of low rates of inbreeding on the variability, indicating the extent to which natural selection may retain heterozygosity; (*b*) the extent to which selected lines return to their original point after selection for a few generations (that is, before many genes are fixed) followed by relaxation; (*c*) the fitness of lines selected artificially for a few generations.

In Edinburgh, I and some of my colleagues have been carrying out a many-sided attack on this problem. The results are still coming in and it is still far too soon to attempt to summarize them because we are not even convinced ourselves of what they mean. However, it seems clear that, as far as bristles are concerned, the average degree of overdominance for fitness of the "factors" controlling these characters (I deliberately avoid the use of the word "gene" here) is of the order of 1 or 2 per cent. This probably implies that the great majority of the genes concerned are effectively neutral in fitness.

The second aspect of this problem—the evolutionary shaping of the mean and variability of the separate measurements which make up the individual, is exceedingly complex. I suspect that one of the difficulties lies in the fact we are attempting to tackle the problem of causality like nineteenth-century physicists—by pretending that we can isolate and manipulate the different characters independently. In a sense the problem is rather similar to that of the economist who, despite the possession of a multitude of data on the national economy, still finds it extraordinarily difficult to predict the immediate future with any accuracy. Like the geneticist dealing with many measurements on the individual, almost all the things the economist can observe are at the same time partly cause and partly effect, being merely aspects of an exceedingly complex causal process.

Having deliberately decided to cover a wide field in this paper, I have probably dealt with some parts of it far too sketchily to be convincing. I hope, however, that I have convinced you that those of us who devote part of our energies to *Drosophila* and mice are not wasting our time. Of one thing I am sure—that, as a result of my

own work with *Drosophila*, I feel far more competent to discuss improvement of dairy cattle than if I had spent all my time analysing milk records and perhaps breeding a few cattle.

REFERENCES

BATEMAN, N. 1958. Response of mice to selection for lactation. Proc. X Internat. Congr. Genet. *2*: 14.

CLAYTON, G. A., J. A. MORRIS, and A. ROBERTSON. 1957. An experimental check on quantitative genetical theory. I. Short-term responses to selection. J. Genet., Cambr. *55*: 131–151.

FALCONER, D. S. 1952. The problem of environment and selection. Am. Natur. *86*: 293–298.

——— 1955. Patterns of response in selection experiments with mice. Sympos. Quant. Biol. *20*: 178–196.

LERNER, I. M., and E. R. DEMPSTER. 1951. Attenuation of genetic progress under continued selection in poultry. Heredity *5*: 75–94.

MATHER, K., and B. J. HARRISON. 1949. The manifold effect of selection. Heredity *3*: 1–52, 131–162.

ROBERTSON, F. W., and E. C. R. REEVE. 1952. Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in *Drosophila melanogaster*. J. Genet., Cambr. *50*: 414–448.

——— 1955. Studies in quantitative inheritance. VIII. Further analysis of heterosis in crosses between inbred lines of *Drosophila melanogaster*. Zschr. indukt. Abstamm. *86*: 439–458.

ON THE APPLICATIONS OF BLOOD GROUPS IN ANIMAL BREEDING

Clyde Stormont[1]

CONTEMPORARY STUDIES of blood groups in animals can be said to have begun approximately two decades ago in the Immunogenetics Laboratory of the University of Wisconsin, with the reports of Ferguson (1941) and Ferguson, Stormont, and Irwin (1942) on the demonstration and inheritance of thirty blood factors in cattle. Eighteen years have elapsed since the significance of bovine blood-typing was first realized in connection with the identification of individual animals, in ascertaining the validity of registrations, and in solving parentage problems arising in the registration of purebred cattle (Ferguson, 1941; Ferguson *et al.*, 1942; Stormont and Cumley, 1943). Considerable progress has since been made in elucidating the genetic systems of blood groups in cattle (Stormont, 1947, 1952, unpublished 1947–58; Stormont *et al.*, 1951), thereby greatly improving the utility of the blood-typing tests for cattle in the solution of parentage problems (Miller and Stormont, 1957) and many of their other applications. During the same period the practical application of artificial breeding in cattle was developed and it is now feasible to think of breeding cows with semen that has been kept frozen for many years.

Because of the widespread use of artificial insemination in cattle and because of the potential use of frozen semen years after the animals from which it is collected have become deceased, the problems arising in the registration of artificially bred animals have increased. Realizing the great utility of blood grouping in connection with such problems, the Purebred Dairy Cattle Association of America (representing the American Guernsey Cattle Club, the American Jersey Cattle Club, the Ayrshire Breeder's Association, the Brown Swiss Breeder's Association and the Holstein-Friesian Association of America) ruled some years ago that all registered dairy bulls used for artificial insemination must have their blood groups officially recorded. Subsequently, dairy-breed organizations in Canada, the Scandinavian countries, the Netherlands, and other parts of the world have adopted similar rulings and, as a consequence, numerous laboratories for cattle blood-typing have sprung into existence to meet the needs of the industry.

Also, in the same laboratory, Owen and his colleagues (1945, 1946) discovered the remarkable phenomenon of erythrocyte mosaicism in dizygotic cattle twins and higher zygotic multiples. Owen's studies, in conjunction with those of Stormont (1949), on the acquisition of the J substance by the bovine erythrocyte, paved the way for the use of blood-typing tests for the exclusion of freemartinism and monozygosity in twins and higher multiples, both in cattle and in sheep (Stormont *et al.*, 1953).

At the same time Briles and his colleagues (1950a, 1950b, 1951, 1952) made studies of the genetic systems of blood groups in chickens and the use of blood-group loci in measuring residual heterozygosity (Briles *et al.*, 1948, 1957) and combining ability or heterosis (Shultz and Briles, 1953; Briles, 1957). The poultry industry and poultry research stations were quick to realize the great

[1]School of Veterinary Medicine, University of California, Davis, California, U.S.A.

potentialities of chicken blood groups for breeding programs and a number of different investigations are now being undertaken.

More recently, in our laboratory, Sprague (1957, 1958) has discovered a soluble blood-group property called Oc which is related to the J property of cattle, and tests for that property promise to increase the utility of cattle blood-typing in connection with the exclusion of monozygosity in twins and higher multiples. Also, Rasmusen recently completed his dissertation (1958) on genetic systems of blood groups in sheep, a subject which he is discussing at these meetings, and we can expect that his findings may ultimately be put to use in sheep-breeding programs. Studies are also in progress in various laboratories concerning genetic systems of blood groups in horses and swine, but as yet they are much in their infancy. (Beyond the scope of the present discussion are the many recent studies related to blood groups in laboratory animals and various species of fish.)

A recent series of reviews and articles (Braend, 1956; Ferguson, 1955; Irwin, 1956, Neimann-Sorensen *et al.*, 1956; Rendel, 1958a, 1958b) has covered in detail most of the applications of blood-group studies in animal breeding. However, since population geneticists, husbandmen, and others are becoming more and more interested in the use of blood groups in breeding programs, particularly since the reports of Briles and his colleagues appeared, it might be well to devote a considerable portion of our discussion to the subject of the blood groups themselves. And, since the serologic and genetic facts about blood groups are much the same, even in widely separated species, we shall limit our discussion largely to those blood groups found in cattle. There are several good reasons for choosing cattle as our example, chief among them the fact that more is known about genetic systems of blood groups in cattle than is known about those in other species. However, before beginning that discussion, it may be well to review some of the more pertinent serological and genetic facts about blood groups and to say a word about terminology.

Blood-grouping, of course, is concerned to a large extent with the effects of genes on the serologic properties of the discoplasm or envelope of red cells. In general, it is found that genes at several loci affect those properties. The number of such loci has not yet been found to exceed twelve in any one species. The number of alleles per locus ranges from two to upwards of a hundred and sixty (the B locus in cattle) and it was the evidence for the extensive allelic differentiation at certain of the blood-group loci which suggested that individuals heterozygous for such alleles may have selective or adaptive advantages over their homozygous contemporaries.

With the exception of the soluble blood-group substances associated with such systems as A-B-O and Lewis in man, J-Oc in cattle and R-O in sheep, virtually nothing is known concerning the structure of the molecules of the discoplasm that are affected by the action of blood-group genes. Because of the extensive cross or overlapping serological reactions shared by those molecules affected by allelic genes, it can be inferred that such molecules are very much alike, at least with respect to their surface or haptenic structure. On the other hand, the failure to demonstrate cross reactions between or among the molecules affected by most non-allelic genes leads to the inference that such molecules are decidedly different in structure, at least with respect to their surface configurations.

Terminology

We shall refer to the molecules affected by individual genes as *phenogroups* or *phenogroup-molecules* and to the serological reactions of the various blood-typing antibodies (reagents) that characterize phenogroups as *blood factors.*

We must be careful not to convey the impression that the multiple blood factors written into the "long-hand" symbols of phenogroups bear any physical reality as specific, particulate, and independent "unit antigens" or that they each might represent a special combining site for a single kind of antibody. The notion that they do so has been all too prevalent in many of the writings on blood groups, even though the reasoning that has led to such a conclusion has been repeatedly exposed (cf. Stormont, 1955; Owen, these meetings).

Blood Groups in Cattle

In the first phase of the cattle studies (Ferguson, 1941; Ferguson, Stormont, and Irwin, 1942), it was thought that each of the thirty bovine blood factors then recognized represented a specific, particulate, and, for the most part, independent antigenic substance. Each of these hypothetical substances (named A, B, C, E, G . . . etc.) was alleged to be controlled by a single gene, and it was widely presumed that "markers" had probably been provided for most of the thirty pairs of chromosomes in cattle. The number of possible blood types in cattle was said to be 2^{30}, a number which was revised to 2^{40} in order to take into account some additional bovine blood factors, described later by Stormont (1950).

The notion that correlations might be established between various combinations of the cattle blood factors and such things as the level of milk production and the amount of fat an animal might produce (see Ferguson, 1955) attracted considerable attention to the studies. The ultimate goal, as stated by Ferguson, was that of blood-typing calves and predicting their performance as mature animals merely by examining their blood factors. As far as I know, such correlations are yet to be established. However, we can often identify the breed of a calf by inspecting its blood groups, and knowing the breed, we can make reasonably accurate predictions concerning the animal's ultimate performance in producing milk and fat.

Actually, the first combination of bovine blood factors that received more than cursory examination was the combination "BGK," but no attempt was made to establish correlations between BGK and economic traits, although this combination makes its appearance in some of the high butterfat breeds much more frequently than in the low butterfat breeds.

I observed in the first phase of the cattle studies that the blood factors B, G, and K were not distributed in a manner expected of genetic characters presumably controlled by non-allelic genes. On the contrary, B, G, and K were appearing (that is, with respect to one another) in only five of eight expected serotypes. The observed serotypes were BGK, BG, B, G, and "no-BGK" and the missing types, which are still missing, were BK, GK, and K.

We cannot dwell here on the BGK story for that is a long one involving numerous data which may never be published. I should like, however, to mention the impact of BGK on the genetic analysis of blood groups. First and perhaps most significantly, BGK was responsible for emphasizing the concept "phenogroup" and with it the realization that asymmetrical occurrence in combinations among and between blood factors (see W. J. Miller, these meetings) can be accounted for on the basis of *true* serological cross-reactions without resort to any genetic hypotheses whatsoever. Secondly, BGK provided a beginning point, a break-through as it were, in the analysis of genetic systems of blood groups in cattle (Stormont, 1947; Stormont *et al.*, 1951).

The analysis of the inheritance of the serotypes BGK, BG, B, G, and no-BGK

retrieved most of the thirty bovine blood factors, described by Ferguson (1941) and Ferguson *et al.* (1942), from the positions which most of them had assumed as alleged markers of presumably independent loci and placed them in proper systems of blood groups, as shown in Figure 1.

Figure 1 also shows the fate of some thirty additional bovine blood factors, only thirteen of which have so far been described in the literature (Stormont, 1950). Additional systems, named D, L, M and Z′, have been evoked to handle, respectively, blood factors D, L, M (subtypes M_1 and M_2) and Z′. However, the assignment of the systems M and Z′ is still tentative. At present, our family data indicate that the rare blood factors M and Z′ cannot be members of the systems A-H, B, C, F-V, J-Oc, L and Z, but the data do not exclude the possibility that either M or Z′, or both, could be members of the D or S-U system, or of the same system.

Data are presented in Table I relating to the main sources of antisera (column 2) and the number of blood-typing reagents per system (column 3) now in use in cattle blood-typing tests performed in this laboratory. Also shown in Table I are the numbers of phenogroups, genotypes, and distinguishable phenotypes in each of eleven systems as defined by means of our reagents. By combining our estimates of distinguishable phenotypes per system (last column in Table I) we arrive at an estimate of 156,520,000,000 possible blood types in cattle.

TABLE I

BLOOD GROUPS IN CATTLE

Systems	Sources of antisera*	Number of			
		Blood-typing reagents†	Phenogroups	Genotypes	Distinguishable phenotypes‡
A–H	BIA and RHA	3	6	21	6
B	BIA, OIA, and RHA	40	164	12,530	10,000‡
C	BIA, OIA, and RHA	11	35	630	200‡
D	RHA	1	2	3	2
F–V	RHA and BIA	2	2	3	3
J–Oc	NI	2	4	6	4
L	BIA	1	2	3	2
M	BIA	2	3	6	3
S–U	RHA and BIA	6	6	21	15
Z	RHA and BIA	2	2	3	3
Z′	BIA and RHA	1	2	3	2

*BIA (bovine isoimmune antisera); NI (natural isoantibodies); OIA (ovine isoimmune antisera); RHA (rabbit heteroimmune antisera).

†Includes only those reagents developed and in use in this laboratory.

‡As indicated on the basis of reagents in use in this laboratory; the estimates in the B and C systems are only approximations.

PHENOGROUPING

The ultimate goal in blood-typing any individual is direct phenotypic classification of each of the alternative phenogroups in each of the systems. In essence, this amounts to "genotyping," but the process is called "phenogrouping." That goal was reached early in the cattle studies with respect to the systems named F-V and Z, as described in the first published report (Stormont, 1952) on those systems. For example, in the F-V system (Table I) three phenotypes F, FV, and V are recognized and these are controlled by a pair of alleles F^F and F^V. The allele F^F is said to control phenogroup F which is characterized by its reactions with F reagent. The

allele F^V is said to control phenogroup V which is characterized by its reaction with V reagent. The phenogroup formulas are therefore written F/F, F/V, and V/V, corresponding respectively to the genotypes F^FF^F, F^FF^V, and F^VF^V. Similarly, in the Z system there are two principal phenogroups said to be controlled, respectively, by a pair of alleles Z and z. So far, however, it has not been possible to develop a reagent which will react with the product of allele z to the exclusion of the product of allele Z. However, it was possible, very early in these studies, to isolate a special kind of Z reagent which reacted much more strongly with the red cells of homozygotes (ZZ) than with those of heterozygotes (Zz). Consequently, with the aid of these special reagents, now generally referred to as "dosage reagents," it is possible to diagnose in the direct tests the three phenogroup formulas Z/Z, Z/–, and –/–, corresponding, respectively, to the three genotypes ZZ, Zz, and zz.

The systems D, L, and Z′ (Fig. 1, Table I), like the Z system, are so-called "two-allele," "one-blood-factor" systems but, so far, it has not been possible to develop dosage reagents for the blood factors. Consequently, direct diagnosis of the alterna-

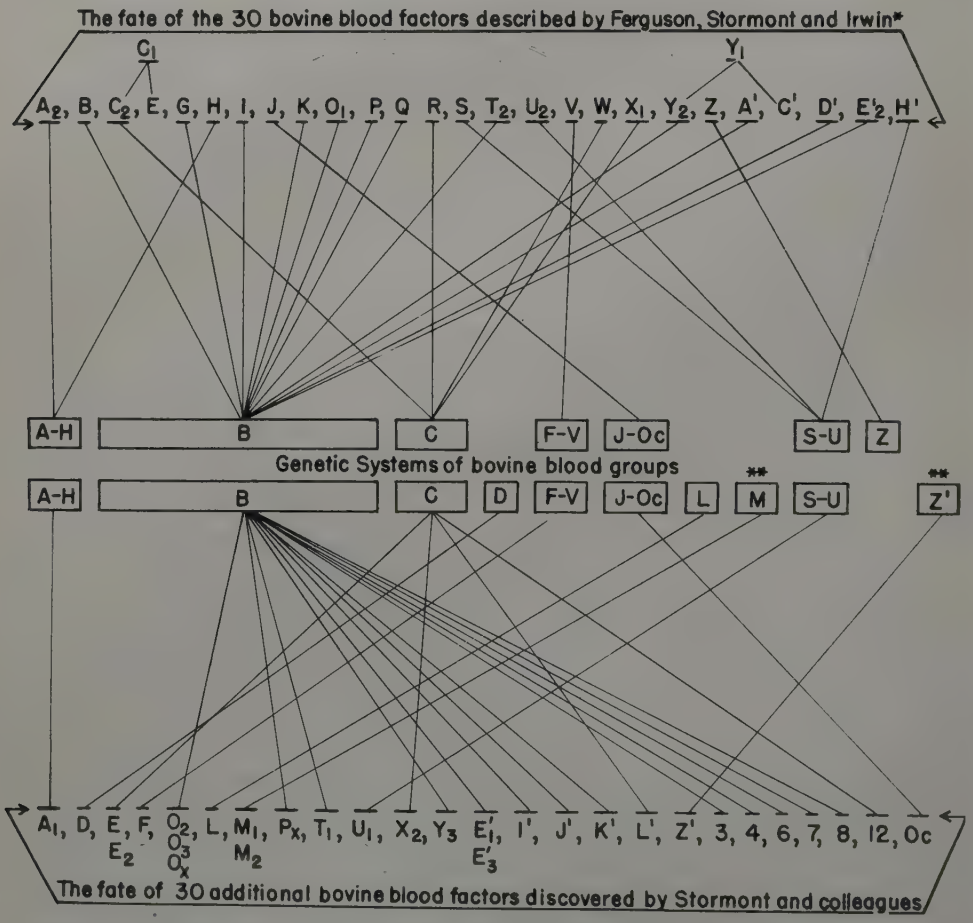


FIGURE 1. *Tests for four of the thirty blood factors, namely, M, N, F1, and G1, were discontinued prior to the beginning of the analysis of the genetic systems of blood groups.
**The assignment of separate systems named M and Z1 is still tentative.

tives in each of those systems is presently not possible. Similarly, as indicated in Table I, direct diagnosis for each of the pairs of alternatives in the systems A-H and J-Oc is presently not possible.

We think rather remarkable progress has been made, particularly within the last few years, in the direct diagnosis of the various paired alternatives (at least 10,000, from data in Table I) in the B system. This has come about mainly by the incorporation in the tests of new varieties of B-system reagents, including the use of ovine isoimmune antisera (Stormont *et al.*, 1957), and the use of reagents that exhibit dosage for many of the blood factors in that system. Then, too, as indicated by the data in Table V, the combinations of blood factors that characterize phenogroups in the B system permit their subclassification into a variety of sets in which singularly unique patterns of combination become clearly evident.

Similarly, rather remarkable progress has been made recently in the direct diagnosis of the pairs of phenogroups in the S-U system (Table I) and it is now possible, particularly with the aid of dosage reagents for blood factor H′, to diagnose, in the direct tests, fifteen of the twenty-one pairs of S-U phenogroups.

Although considerable progress has been made in the direct phenogrouping of the pairs of phenogroups in the C system, we are still some distance from the desired goal, as indicated by the data given in Table I.

The Importance of Phenogrouping

Obviously, we cannot overestimate the importance of direct phenogrouping, not only from the standpoint of virtually all of the applications of blood-grouping tests but also from the standpoint of the standardization and repeatability of the tests so that the results obtained in one laboratory can be utilized in another. Furthermore, direct phenogrouping provides a measure of control over these delicate tests that cannot possibly be approached by other methods of control. False negative and false positive reactions can be spotted immediately.

Applications of Phenogrouping

In order to illustrate some of the applications of phenogrouping and to provide some idea of the immense complexity of the cattle tests, I have selected excerpts from the results of a more or less routine blood-typing test performed in this laboratory and these are given in Tables II and III.

The procedure is such that forty cattle are blood-typed in one routine test. Each blood sample is typed with, on the average, approximately eighty serological reagents. Inclusive of controls, such a test requires the use of 3,280 tubes (10×75 mm.). Into each tube (except the complement controls) are delivered two drops (approximately 0.10 ml.) of a given reagent (which has been properly diluted), one drop of a 2.5 per cent suspension of the washed erythrocytes of the animal being tested, and one drop of complement (fresh rabbit-serum), in that order. The tubes are shaken just prior to the addition of complement and again following the addition of complement.

The tests are performed at room temperatures in the range of 23 to 28°C. and, in our experience, should not exceed the two extremes. Three readings are made, the first beginning at approximately half an hour after the complement is added, the second one and a half hours after the first and the third one and a half hours after the second. The tubes are shaken again after the first and second readings. The first

TABLE II

EXCERPTS FROM A SUMMARY OF A BOVINE BLOOD-TYPING TEST PERFORMED ON 40 BLOOD SAMPLES*

Tests with reagents reacting in the blood-group systems

Code no. of test cells	A-H			B																										
	A_1	A_2	H	B_1	B_2	G	I	K	O_1	O_3	O_x	P	P_x	Q	T_1	Y_1	Y_2	Y_2†	A′	D′	E_1′	E_2′	E_3′	I′	J′	K′	K′†	4	6	7
H2	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	+	±	+	0	0	0	0	0	0	+	0	0	0	0
H4	0	0	0	0	+	+	0	0	0	0	0	0	+	0	0	+	+	+	0	+	+	+	+	0	0	0	0	0	0	0
H7	0	0	0	+	+	+	0	+	0	0	+	0	+	0	0	0	+	±	+	0	+	+	+	0	0	0	0	0	0	0
H19	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H20	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	+	+	±	0	+	0	0	0	+	0	0	0	0	0	0
H21	0	0	0	0	0	+	0	0	0	0	0	0	+	0	0	0	+	+	0	0	+	+	+	0	0	0	0	0	0	0
G33	+	+	+	+	+	+	0	+	0	0	+	0	+	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	+
G34	+	+	+	+	+	+	0	+	0	+	+	0	+	0	0	0	0	0	+	0	0	+	+	0	0	+	±	0	+	+
G35	+	+	+	+	+	+	0	+	0	+	+	0	+	0	0	0	0	0	+	0	0	+	+	0	0	+	±	0	+	+
G36	+	+	+	+	+	+	0	+	+	+	+	0	+	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0	0	+

Code no. of test cells	B				C									D	F–V		J–Oc			L	M	S–U					Z		Z′
	8	S5†	S26†	S34	C_1	C_2	E	E_2	R	W	X_1	X_2	L′	12	D	F	V	J	J‡	Oc‡	L	M	S	U_1	U_2	H′	H′†	Z	Z†	Z′
H2	0	0	0	0	0	0	0	+	0	0	+	+	0	+	+	+	0	+	+	+	0	0	0	0	0	+	0	0	0	0
H4	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	+	0	0	0	0	0	+	±	+	±	0
H7	+	0	0	+	0	0	0	0	0	0	0	+	0	+	+	+	0	0	0	+	0	0	0	+	+	+	±	0	0	0
H19	0	0	0	0	0	0	+	+	0	+	0	+	0	+	+	+	0	+	+	+	+	0	0	0	0	+	+	0	0	0
H20	0	0	0	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	0	0	0	+	+	+	±	0
H21	0	±	+	0	0	0	+	+	0	0	0	+	0	+	+	+	0	0	+	+	0	0	0	0	0	0	0	0	0	0
G33	+	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	0	+	0	0	+	+	0	0	0
G34	+	±	0	+	+	+	0	+	0	+	0	0	0	0	+	0	+	+	+	+	+	0	+	0	0	+	±	0	0	0
G35	+	±	0	+	0	0	+	+	0	+	0	0	0	0	+	0	+	+	+	+	+	0	0	0	0	+	±	0	0	0
G36	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	0	0	0	+	±	0	0	0

*Tested January 17, 1958.

†Dosage reagents.

‡Inhibition tests for soluble J and Oc properties in blood plasma.

TABLE III

A Summary of the Phenogroups and Probable Phenogroup Formulas in the Animals Represented in Table II

Code no.	Genetics systems of blood groups†											
	A–H	B*	C	D	F–V	J–Oc	L	M	S–U	Z	Z′	Relationship
H2	−/−	B61/B101	E_2/X_1	D	F/F	JOc	−/−	−/−	H′/−	−/−	−/−	Dam of H19
H4	−/−	B38/B39	C_1/WX_2	D	F/F	JOc	−/−	−/−	H′/−	Z/−	−/−	Dam of H20
H7	−/−	B24/B39	$X_2/$	D	F/F	Oc	−/−	−/−	U_1H′/−	−/−	−/−	Dam of H21
H19	A_1	b/b	E/WX_2	D	F/F	JOc	L	−/−	H′/H′	−/−	−/−	Daughter of H2
H20	−/−	B38/B89	E/WX_2	D	F/F	± JOc	−/−	−/−	H′/H′	Ż/−	−/−	Daughter of H4
H21	−/−	B39/B39	E/X_2	D	F/F	± JOc	−/−	−/−	−/−	−/−	−/−	Daughter of H7
G33	A_1H	B12/B12	$C_1E_2WX_2$	D	F/F	JOc	L	−/−	SH′/H′	−/−	−/−	Dam of G34, G35, & G36
G34	A_1H	B12/B20	C_1E_2W	−/−	F/F	JOc	L	−/−	SH′/−	−/−	−/−	Daughter of G33
G35	A_1H	B12/B20	EW	−/−	F/F	JOc	L	−/−	H′/−	−/−	−/−	Daughter of G33
G36	A_1H	B12/B47	$C_1E_2WX_2$	−/−	F/F	JOc	−/−	−/−	H′/−	−/−	−/−	Daughter of G33

*See Table V for codes and decodes of phenogroups in the B system.
†The diagonal line (/) is used to separate a pair of phenogroups.

reading is performed at a time when the unaffected (that is, non-hemolysed) red cells are still in suspension and the main purpose of this so-called "early" reading is to record differences in rates of hemolysis, especially with respect to the dosage reagents.

The summary shown in Table II is a composite of the three readings of that particular test. The + (plus) symbol indicates a positive reaction (hemolysis) and the 0 (zero) symbol a negative reaction. The ± symbol is used primarily in connection with dosage reagents to indicate that there was a positive reaction but that the reaction was indicative of only one dose of the given blood factor, as opposed to two as indicated by the + symbol. Some of the reagents, particularly the reagents labelled S5 and S26 (ovine isoimmune antisera), exhibit dosage with particular combinations of phenogroups rather than with individual blood factors. For example, the reagent labelled S5 reacts to some degree (±) with the red cells of animals homozygous for phenogroup B39 (see Table V for decode) but usually not at all with the red cells of individuals heterozygous for B39.

Table III shows a summary of the phenogroups indicated by the results given in Table II. In the systems A-H, D, J-Oc, L, M, and Z′, where direct phenogrouping of both alternatives is not possible, the accepted procedure in the cattle studies is to list the blood factors (positive reactions) where they occurred and to use the symbol -/- to indicate negative reactions with all the reagents used in typing a given system. The ± symbol above blood factor J in the J-Oc system (test cells numbered or coded H20 and H21) indicates that the red cells were not hemolysed but that the blood plasma of those individuals was capable of inhibiting the reactions of J antibodies.

The blood samples involved in the test from which the data in Tables II and III were obtained had been sent to this laboratory for analysis in connection with a project on dairy cattle blood-typing repeatability and standardization being sponsored by the Dairy Husbandry Research Branch (DHRB) of the Animal Husbandry Research Division of the United States Department of Agriculture (Anon., 1957). Replicas of the same forty samples were also sent to each of six other laboratories (two in United States and four in Europe).

The procedure in each of the "40-cell trials" calls for each of the participating laboratories to report its results in accordance with the form given in Tables II and III. After the DHRB has received the results in any one trial from all the participating laboratories, the laboratories are then provided with information on the relationship of the animals involved in that trial. The results shown in Tables II and III are the results on ten animals in the fourth trial which were said to be related as parent and offspring. The stated relations are as given in the last column of Table III.

By comparing the phenogroup formulas of the parent-offspring pairs numbered H4-H20, H7-H21, G33-G34, G33-G35, and G33-G36, we note that there is genetic compatibility between the proposed pairs of phenogroups in parent and offspring in each of the critical systems B, C (as far as phenogrouping was done), F-V, S-U, and Z. However, with respect to the pair H2-H19 we note that neither of the members of the proposed pairs of phenogroups in the B and C systems of the dam (H2) made its appearance in the blood of the alleged daughter (H19). The results constituted evidence for an unconditional exclusion of maternity. H2 could not be the dam of H19 regardless of who the sire might be.

As it turned out, the proposed sires of each of the six daughters listed in Table III had been blood-typed in our laboratory in connection with service-typing work done

for member organizations of the Purebred Dairy Cattle Association of America. The phenogroups of those sires are recorded in Table IV and the stated relationship of those sires to the daughters listed in Table III is shown in the last column of Table IV. By comparing the phenogroup formulas of each of the sire-daughter pairs, we note that there is good agreement between the proposed phenogroups of the sires and those in their respective daughters, including H19, the alleged daughter of cow H2 and bull no. 846425. Consequently, at this point in the analysis we could say that bull 846425 qualifies as the sire of H19 in spite of the fact that cow H2 is clearly excluded as the dam.

The next step was to compare the phenogroups in H2 (the alleged dam of H19) with those of her proposed parents, and in doing so it was clear that neither of the B phenogroups of the proposed sire of H2 and neither of the B phenogroups of the proposed dam of H2 made their appearance in H2, thereby constituting evidence for a bilateral exclusion of parentage.

We, therefore, arrived at the tentative conclusion that the blood sample labelled H2 had been drawn, by mistake, from an animal not related as the dam of H19. If this conclusion were correct, the next obvious step would be that of attempting to establish the identity of the animal from which the sample labelled H2 had been drawn. It was possible that one of the animals sampled in an earlier trial might have been the source of the sample labelled H2, and, although our laboratory had not participated in the first two trials, we fortunately had available the results of the tests reported by the Immunogenetics Laboratory of the University of Wisconsin. By comparing the phenogroup formula of H2 with those of other animals sampled in the various trials, much in the manner that one might compare dermatoglyphs, it was possible to find one and only one animal out of a total of 159 samples tested that matched the phenogroups of H2. The matching blood had been drawn from an animal sampled in the first trial and we were relatively certain at this point that the sample labelled H2 had in fact been drawn by mistake from that animal. As it turned out, that conclusion was correct. The true dam of H19 had not been sampled, and when the blood sample from the real H2 arrived and was tested it was clear that the true dam of H19 had at long last been found.

Now I suppose the moral of the story is, "Never trust a label on a bottle," but, aside from that moral, we believe our analysis of the foregoing problem combines the essential elements of the use of phenogrouping in the solution of parentage problems as well as in the establishment of the identity of individual animals, and that is why we chose that particular example. You may ask, "Could we not have established the identity of the sample labelled H2 simply by comparing blood types?" The answer is, "No." The reason is that no two laboratories ever test with precisely the same reagents. Furthermore, two animals which have the same blood types can differ with respect to the pairs of phenogroups they may have in such systems as B, C, and S-U. You may also ask, "Could we not have questioned the alleged parent-offspring relationship between cow H2, bull 846425, and daughter H19 simply by comparing their blood types?" The answer, once again, is "No." Daughter H19 possessed no known blood factors not represented in the blood of the alleged parents. Actually, exclusive use of the antedated "blood-factor" method of analysis would have permitted the erroneous conclusion that H2 and 846425 qualify as parents of H19. Indeed, phenogrouping clearly exposes the fallacy in the exclusive use of that antedated method of parentage analysis. As pointed out to me by my colleague, Dr. W. J. Miller, the factor method of analysis is based on the erroneous premise that the alternative to any given blood factor is its absence. Both

TABLE IV

PHENOGROUPS AND PROBABLE PHENOGROUP FORMULAS OF FOUR REGISTERED DAIRY BULLS

Genetic systems of blood groups

Bulls	A–H	B*	C	D	F–V	J‡	L	M	S–U¶	Z	Z′	Relationship to animals in Tables II and III
846425	A_1	B3/b†	E/W†	D	F/F	J/−†	L/−†	(NT)″	H′/H′	−/−	−/−	Sire of H19
956081	−/−	B39/B89	E	D	F/F	−/−	−/−	−/−	H′	−/−	−/−	Sire of H20
975138	A_1/−†	B39/B39†	C_2EW/E†	D	F/V	−/−	−/−	−/−	H′/−†	Z/−	−/−	Sire of H21
391988	A_1	B20/B47	C_1EW	−/−	F/F	−/−	−/−	−/−	−/−	−/−	−/−	Sire of G34, G35, and G36

*See Table V for codes and decodes of B phenogroups.
†The alternatives shown were confirmed by a study of the blood groups in known offspring.
‡Tests were not performed for the soluble properties J and Oc in the blood plasma.
″NT (no test).
¶The samples 956081, 975138, and 391988 were not tested with the H′ dosage reagent.

the exclusive use of that method and the erroneous premise are holdovers from the days when each of the bovine blood factors was assumed to be a specific and particulate "cellular character." We can regard any conclusions based on that assumption as somewhat suspect at the outset.

Phenogroups and Twins

Rendel (1958b) has given us a particularly good account of the use of bovine blood groups in the studies of twins. I should, however, like to call attention once again to the advantages of the phenogroup method of analysis in the studies of the bloods of twins. In that particular method, the B system turns out to be the key system, for any results of the direct blood-typing tests that require the postulation of more than two B phenogroups to explain all the B blood factors in the blood of any given animal are indicative of erythrocyte mosaicism or blood admixture. Indeed, it was the necessity of postulating the presence of three B phenogroups in one blood sample that led to the discovery of erythrocyte mosaicism in an animal rcorded as single-born (Stormont, 1954). As is generally known now, the demonstration of parallel mosaics in a set of twins constitutes evidence that the twins came to share each other's genetically and serologically dissimilar cells through chorionic vascular anastomosis. Consequently, it is concluded that such twins are dizygotic. Nevertheless, that conclusion is not infallible. It is not improbable that some monozygotic twins are survivors of sets of dizygotic triplets, or the like, and, as a consequence of anastomosis among such sets, the surviving monozygotic twins would exhibit mosaicism.

Phenogroups and Breed Structure

Some years ago, Owen, Stormont, and Irwin (1947) reported on the frequencies of the thirty blood factors of Ferguson *et al.* (1942) in Guernsey and Holstein-Friesian cattle. They found that the two breeds differed significantly with respect to the frequencies of the majority of those thirty blood factors. Although there were no published reports of the genetic systems of bovine blood groups at that time, Owen and his colleagues were, of course, aware that such systems existed. "The situation in cattle," they wrote, "is, however, somewhat complicated by the existence of 'allelic series' in which individual alleles seemingly control the simultaneous production of more than one antigen [that is, the blood factors of the present report] . . . , etc. Thus, the frequency of an antigen [blood factor] in a complex [phenogroup] of this sort [that is, like those in Tables V and VI] is not a measure of the frequency of a particular 'allele,' but reflects the frequency of all 'alleles' having the elaboration of this antigen [blood factor] in common." Given data like those in Tables V and VI of the present report, we are in a better position to understand this comment. For example, in Table V we note that blood factor K is represented in at least eight phenogroups (B12, B19, B20, etc.).

Owen and his co-workers also stated that a report in preparation by Stormont *et al.* indicated that breed differences in individual "allele" frequencies appear to be even more extreme than those evident from the studies of the frequencies of the individual blood factors. The data in Tables V and VI are presented in support of that statement.

The report referred to above was that of Stormont, Owen, and Irwin on the B and C systems of bovine blood groups (1951). Although that report contained no data

on estimates of the frequencies of individual B and C alleles, it did show that marked differences exist among breeds with respect to the distribution of those alleles and it also pointed out that the marked differences among lines within breeds with respect to those alleles posed a major problem in obtaining samples representative of all the animals within a breed. That problem, we believe, is now being solved by our recent participation in the service-typing work being done for member organizations of the Purebred Dairy Cattle Association of America. Through that program we are receiving blood samples from all registered dairy bulls being brought into artificial breeding units throughout the United States. It is our plan to collect such data over a five-year period before readying them for publication. In the interim we offer the data in Tables V and VI in the hope that they will satisfy more immediate needs.

TABLE V

71 Representative Phenogroups in the B System of Bovine Blood Groups showing Allele Frequencies in 5 Breeds of Cattle in United States

Phenogroups		Breeds‡				
Code	Decode	G	H	F	J	S
K Set						
B12	$BGKO_xE'_27,8$	13	0	2	0	6
B19	$BGKO_xA'K'6,8$	0	0	0	6	0
B20	$BGKO_xA'K'6,7,8$	2	0	0	0	0
B24	$BGKO_xY_2A'8$	0	0	<1	0	0
B25	$BGKO_xA'E_3'8$	0	0	3	0	<1
B26	$BGKO_xY_1A'E'_36,8$	*	0	<1	0	0
B27	$BGKO_xY_1A'E'_3I'6,7,8$	4	0	0	0	0
B28	$BGKO_xY_1A'E'_3K'4,6,8$	11	0	0	24	0
T_1 Set						
B53	$O_1T_1E_3'I'$	*	0	0	0	
B54	$O_1T_1E_3'K'$	4	0	<1	18	0
B56	$O_1T_1E_3'I'K'$	3	0	0	0	0
B57	$O_3T_1E_3'6$	*	0	0	<1	0
B73	T_16	1	0	0	0	0
B74	T_1E_3'	0	0	0	12	0
B75	$O_xT_1Y_1E_3'6$	<1	0	0	0	0
B98	$BO_xT_1E_1'8$	*	0	0	0	0
I Set						
B2	BI	5	0	0	0	0
B23	$BGIO_1T_2A'$	0	0	*	0	0
B30	B_2GI	1	0	2	0	0
B42	I	*	0	*	0	0
B43	IO_xE_1'	23	0	*	0	0
B44	IQE_1'	4	0	0	0	0
B95	IY_1	0	2	0	0	0
B96	I7,8	0	0	<1	0	0
J′ Set						
B60	$O_3J'7,8$	0	0	<1	0	0
B62	$O_3J'K'7,8$	0	0	6	*	0
B63	$O_3Y_2J'K'7,8$	0	0	*	0	0
B64	$O_3Y_1D'J'K'7,8$	0	0	0	*	0

TABLE V (*continued*)

Phenogroups		Breeds‡				
Code	Decode	G	H	F	J	S
PQ Set						
B65	P	0	<1	<1	0	0
B67	PY_2	0	0	<1	0	0
B68	PI′	0	0	<1	0	<1
B69	PQI′	1	0	0	0	0
B70	QI′	0	7	0	0	0
B72	$QE_1'I'$	0	0	<1	0	0
B91	Q	0	*	*	*	0
D′ Set						
B16	BO_1Y_2D'	0	0	4	0	<1
B33	B_2GD'	0	0	0	4	0
B38	B_2GY_1D'	0	0	1	0	0
B71	$QD'E_1'$	*	0	0	<1	0
B77	O_xY_1D'	*	0	0	4	0
B80	Y_1D'	0	<1	0	0	0
B81	$Y_1D'I'$	0	38	0	0	0
B84	$O_xD'E_3'8$	0	0	6	0	0
E′ Set						
B39	GY_2E_1'	0	0	21	0	1
B40	$GY_2E_1'I'$	0	0	*	0	0
B78	$Y_2E_1'4$	0	0	*	0	*
B85	E_1'	*	0	3	3	0
A′ Set						
B17	$BO_2A'E_3'$	0	0	1	0	0
B22	$BO_2Y_1A'E_3'$	0	0	3	0	42
B46	O_1A'	0	0	5	0	0
B49	O_1Y_2A'	*	0	<1	0	0
B76	$O_xY_1A'4$	0	*	1	*	0
B92	O_xA'	0	10	<1	0	<1
B101	O_xY_2A'	0	0	*	0	0
O_1 Set						
B3	BO_1	*	0	4	0	*
B5	BO_16	0	0	<1	0	0
B14	BGO_1Y_1	*	0	0	0	0
B31	B_2GO_1	2	0	<1	0	0
B34	$B_2GO_1Y_2$	0	0	<1	0	3
B45	O_1	<1	<1	<1	2	0
B47	O_1I'	11	0	0	0	0
B50	$O_1Y_1E_3'$	0	0	2	0	0
B109	$O_1Y_1K'4$	0	<1	0	0	0
Residual Set						
b	—	*	31	15	0	42
B4	BI′	<1	0	*	6	0
B29	B_2G8	3	*	0	0	0
B61	O_xK'	0	0	2	0	0
B79	$O_xY_1E_3'4$	0	0	2	0	0
B87	O_xE_3'	0	0	6	0	0
B89	I′	<1	10	3	3	2
B90	$O_xE_3'8$	10	1	3	14	0

*Allele present in breed but not represented in animals sampled.
‡G (Guernsey); H (Hereford); F (Holstein-Friesian); J (Jersey); S (Milking Shorthorn).

TABLE VI

FREQUENCIES OF ALLELES CONTROLLING PHENOGROUPS IN THE SYSTEMS C, A–H, D, F V, J, L, M, S U, Z, AND Z′ IN THREE BREEDS OF DAIRY CATTLE

	C																								
Breeds	— (no — C)	C_1	C_1E	C_1EW	C_2EW	C_1EWX_2	C_1X_1	C_1X_2	C_1RW	C_2	C_2E	C_2ER	C_2X_1	C_2ERX_1	E	EW	W	EL′	RWX_2	WX_2	WX_1	X_1	X_2	X_2L'
Holstein-Friesian	13.5	1.3	17.1	1.3	1.7	0.3	1.7	0.2	0.2	1.2	1.1	1.1	0.0	0.0	19.0	7.2	0.9	0.6	1.9	2.5	0.0	5.1	14.6	7.2
Guernsey	40.0	0.0	3.1	5.6	3.1	17.5	3.1	0.0	0.6	0.0	0.6	0.6	0.0	0.0	0.0	5.0	0.0	0.6	0.0	1.3	3.1	2.5	12.0	1.2
Jersey	0.0	0.0	1.2	25.6		0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	4.4	0.7	40.0		0.6	0.0	19.4	0.0	2.5	3.8	0.0

	A–H					D		F–V		J–Oc†		L		M		S–U‡					Z		Z′	
	—	A_1	A_2	H	A_1H	—	D	F	V	—	J	—	L	—	M*	—	SH′	U_1H'	U_2	H′	—	Z	—	Z′
Holstein Friesian	69	21	2	8	1	2	98	87	13	66	34	82	18	94	6	38	10	6	1	45	68	32	100	0
Guernsey	52	34	5	1	8	19	81	91	9	69	31	58	42	100	0	42	54	2	0	2	50	50	97	3
Jersey	22	24	0	0	54	49	51	63	37	62	38	88	12	95	5	19	26	1	0	54	60	40	98	2

*Includes subtypes M_1 and M_2.

†The soluble blood-group property Oc was not tested for in the populations sampled for this analysis.

‡The allele for the phenogroup SU_2H' was not represented in the animals sampled. Similarly, the allele for the phenogroup A_2H was not represented in the animals sampled.

The data on allele frequencies in Tables V and VI are based on rather small samples. Those for the breeds Holstein-Friesian, Guernsey, and Jersey are based, respectively, on samples of 200, 80, and 80 bulls in artificial breeding units in states west of the Mississippi River and those samples came into this laboratory during the years 1952–3. The Hereford samples (110 in all) were obtained largely from animals in the herds of the University of California. The Milking Shorthorn samples (146) were obtained from the Innisfail Herd owned by John Rowe and Sons of Davis, California. While the samples from the artificial breeding units can be regarded as reasonably random, those from the Herefords and Milking Shorthorns are not. Nevertheless, we are certain that the estimates on the principal B alleles in each of the Hereford and Milking Shorthorn breeds are not greatly biased by the nature of the sampling and the small number of animals sampled. With regard to the samples obtained from males alone, we should point out that we have no reason, as yet, to believe that the frequencies of the alleles differ significantly in the sexes.

The basis for the arrangement of the B phenogroups (Table V) in sets (the K set, the T_1 set, and so on) is given in another report (Stormont, 1955) and will not be reviewed here.

We note, particularly with respect to the data in Table V, that the breeds of cattle are rather sharply delimited, not only with respect to the different phenogroups represented in any breed but also with respect to the frequencies of the alleles controlling the individual phenogroups. This delimitation is emphasized by the arrangement of the B phenogroups in the various sets. We note, for example, that the alleles represented by phenogroups in the K, T_1, and I sets occupy approximately 70 per cent of the B locus in Guernseys and those in the K and T_1 sets occupy approximately 50 per cent of the B locus in Jerseys. In contrast, the alleles represented by phenogroups in the K, T_1, and I sets occupy less than 7 per cent of the B locus in Herefords, Holstein-Friesians, and Milking Shorthorns. Although the Jerseys and Guernseys (both Channel Island breeds) are much alike with respect to some of their principal B alleles (B^{28} in the K set, B^{54} in the T_1 set and B^{90} in the residual set), they differ considerably with respect to others, such as B^{74} in the T_1 set, B^{43} in the I set, B^{33} in the D′ set, and B^{47} in the O_1 set.

The ease with which the breed of an animal can usually be determined by merely examining its B phenogroups may be illustrated by comparing the B phenogroups in the animals in Tables III and IV with the list in Table V. The samples coded H2-H21 were from Holstein-Friesians and those coded G33-G36 were from Guernseys. The first three samples in Table IV were from Holstein-Friesians and the fourth was from a Guernsey. Except for sample H19, there is little difficulty in identifying the breeds on the basis of B phenogroups alone.

Although marked differences among breeds may be noted (Table VI) with respect to the frequency of alleles controlling phenogroups in each of the systems A-H, C, D, F-V, L, M, S-U, Z, and Z′, those differences are not as extreme or delimiting as the differences indicated for B alleles. Nevertheless, the three populations in Table VI are rather well characterized by the different frequencies of those genes. (Earlier estimates of the frequencies of the alleles at the F-V and Z loci (Stormont, 1952) are in close agreement with those given in Table VI.)

Neimann-Sorensen, Sorensen, Andresen, and Moustgaard (1956) have recently reviewed the Danish investigations on blood groups in cattle and have shown that three Danish breeds (Red Danish, Black and White, and Jersey) differ significantly with respect to the B alleles they possess. They pointed out that the Danish Jerseys are similar to American Jerseys with respect to the B alleles they possess. Also, the

three Danish breeds differed significantly with respect to genes at most of the other loci (A-H, F-V, J-Oc, L, Z, Z′, and S-U). The Danish Jerseys were much like American Jerseys with respect to those genes. Similarly, Rendel (1958c) has examined the frequency of blood-group genes in three Swedish breeds (Swedish Red and White, Friesian, and Polled). Here, again, the same order of differences among the breeds is noted.

Phenogroups and Heterosis

Obviously, the large number of alleles segregating at such loci as B and C in cattle, and A and B in chickens (Briles *et al.*, 1950a, 1950b) would suggest that the extensive allelism must be associated in one way or another with heterosis. Much of the work of Briles and his colleagues during the past decade has been devoted to a test of that hypothesis. Indeed, as they (1948, 1957) have shown in virtually every inbred line of chickens so far examined both A alleles and B alleles are still segregating. Furthermore, the studies of Shultz and Briles (1953) and Gilmour (1958) have shown that artificial selection favors birds heterozygous at the A and B loci. Virtually all of the important economic traits (hatchability, viability, and egg production) seem to be favorably influenced by heterozygosity, particularly with respect to the B alleles, and Shultz and Briles (1953) noted favorable interaction between the A and B loci with respect to some of these traits. Briles (1957) has given us a rather thorough discussion of the possible manner in which the B locus in chickens might exert its heterotic effect and, in that connection, he has also discussed the rash of reports relating the ABO agglutinogens (phenogroups) of man to carcinoma, ulcers, diabetes, and the like.

The question remains whether the findings of Briles and co-workers will be generally applicable in other species. I am certain that cattle-breeders would be particularly anxious to know whether artificial selection favors the selection of cattle that are heterozygous for the various blood-group genes. In examining the data on blood groups in numerous sire-families in various herds and breeds, I have yet to find any significant evidence that daughters heterozygous for alleles at the B, C, F-V, and Z loci are being favored over their homozygous contemporaries. On the contrary, the observed ratios of homozygotes to heterozygotes are almost always closely similar to the expected. On the other hand, Laben and Stormont (1958) have recently completed an analysis of blood groups and various traits in 270 cattle in the University of California inbred herd of Jerseys and have found some evidence, albeit little, that heterozygotes at the B locus may occasionally outperform their homozygous contemporaries. In one sire group, twelve daughters heterozygous for phenogroup B77 (D′ set of Table V) yielded significantly more FCM (fat corrected milk) than nine homozygous (B77/B77) daughters of similar inbreeding. In another sire group, closely related to the former, a significant difference in reproductive efficiency, favoring the B77 heterozygote, was found in a like comparison of nine to ten daughters. The chief difficulty is the present lack of appropriate material (inbred lines and the like) in which to put the hypothesis to rigorous test.

There are many experiments which could be performed utilizing the cattle blood-group loci. For example, it is desirable to know the effects of forced homozygosity and forced heterozygosity at the blood-group loci during the course of inbreeding experiments. It is also desirable to know whether particular heterozygous combinations of B alleles or C alleles or S-U alleles will indicate favorable combining ability

in crossing inbred lines and the like. These and many other questions will be answered in the generations to come. Meanwhile, the time seems ripe to begin such experiments.

In my opinion such experiments will lead to more valuable information than will those which attempt to establish correlations between combinations of blood factors and economic traits. Such correlations will be established just like the establishment of correlations between blood factor A of man and stomach carcinoma. On the other hand, the obvious meaning of many of those correlations will become evident by inspecting data like those given in Tables V and VI. Certainly blood factor K (or the combination BGK) is positively correlated with high butterfat in cattle and perhaps the chief reason for this is the fact that such alleles as those controlling the phenogroups B12, B19, B27, and B28 have a rather high frequency in the high butterfat breeds (Jersey and Guernsey). Conversely, the allele *b* (residual set) has a low frequency in Jerseys and Guernseys and a rather high frequency in the low butterfat breeds (Hereford, Holstein-Friesian, and Milking Shorthorns). We might conclude, therefore, that the null or negative combination of blood factors in the B system tends to depress the amount of butterfat in milk. A parallel trend carries over to other genetic systems of blood groups (Table VI). For example, the combined incidence of alleles associated with blood factor A in Holstein-Friesians, Guernseys and Jerseys is, respectively, 0.24, 0.47, and 0.78. McClure (1952), however, was not able to demonstrate a difference between A-positive and A-negative Friesians with respect to the percentage of butterfat in their milk.

REFERENCES

Anonymous. 1957. Blood will tell. Agr. Res. USDA *6* (3): 8–9.

Braend, M. 1956. The use of blood groups in bovine disputed parentage cases. Cornell Vet. *46*: 83–87.

Briles, W. E. 1951. A new blood group in chickens. Poultry Sc. *30*: 907–908 (Abstract).

——— 1957. The effect of B blood group system on 10-week weights of chicks resulting from a cross between inbred lines. Poultry Sc. *36*: 1106 (Abstract).

Briles, W. E., C. P. Allen, and T. W. Millen. 1957. The B blood group system of chickens. I. Heterozygosity in closed populations. Genetics *42*: 631–648.

Briles, W. E., C. O. Briles, and J. H. Quisenberry. 1950. Three loci affecting the blood group antigens of the chicken. Poultry Sc. *29*: 750 (Abstract).

Briles, W. E., R. W. Briles, and M. R. Irwin. 1952. Differences in specificity of the antigenic products of a series of alleles in the chicken. Genetics *37*: 359–368.

Briles, W. E., and W. H. McGibbon. 1948. Heterozygosity of inbred lines of chickens at two loci effecting cellular antigens. Genetics *33*: 605 (Abstract).

Briles, W. E., W. H. McGibbon, and M. R. Irwin. 1950. On multiple alleles effecting cellular antigens in the chicken. Genetics *35*: 633–652.

Ferguson, L. C. 1941. Heritable antigens in the erythrocytes of cattle. J. Immun., Balt. *40*: 213–242.

——— 1955. The blood groups of animals. *In* Advances in Veterinary Science, vol. II, pp. 106–137. Ed. C. A. Brandly and E. L. Jungherr. New York: Academic Press.

Ferguson, L. C., C. Stormont, and M. R. Irwin. 1942. On additional antigens in the erythrocytes of cattle. J. Immun., Balt. *44*: 147–164.

Gilmour, D. G. 1958. Maintenance of segregation of blood group genes during inbreeding in chickens. Heredity *12*: 141–142. (Abstract).

Irwin, M. R. 1956. Blood grouping and its utilization in animal breeding. VII Internat. Congr. Animal Husb., Madrid *2*: 7–41.

Laben, R. C., and C. Stormont. 1958. Genetic analysis of the B, F-V and Z blood-group loci in an inbred Jersey herd. J. Animal Sc. *17*: 1139–1140 (Abstract).

McClure, T. J. 1952. Correlation study of bovine erythrocyte antigen A and butterfat test. Nature *170*: 327.

MILLER, W. J., and C. STORMONT. 1957. Parentage exclusions by blood grouping in dairy cattle. Genetics *42*: 385–386 (Abstract).

NEIMANN-SORENSEN, A.; P. H. SORENSEN; E. ANDRESEN; and J. MOUSTGAARD. 1956. VII Internat. Congr. Animal Husb., Madrid *2*: 87–111.

OWEN, R. D. 1945. Immunogenetic consequences of vascular anastomoses between bovine twins. Science *102*: 400–401.

OWEN, R. D., H. P. DAVIS, and R. F. MORGAN. 1946. Quintuplet calves and erythrocyte mosaicism. J. Hered. *37*: 291–297.

OWEN, R. D., C. STORMONT, and M. R. IRWIN. 1947. An immunogenetic analysis of racial differences in dairy cattle. Genetics *32*: 64–74.

RASMUSEN, B. A. 1958. Blood groups in sheep. Doctoral dissertation, Univ. California.

RENDEL, J. 1958a. Studies of cattle blood groups. II. Parentage tests. Acta agr. scand. *8*: 131–161.

——— 1958b. Studies of cattle blood groups. III. Blood grouping as a method of diagnosing the zygosity of twins. Acta agr. scand. *8*: 162–190.

——— 1958c. Studies of cattle blood groups. IV. The frequency of blood group genes in Swedish cattle breeds, with special reference to breed structure. Acta agr. scand. *8*: 191–215.

SHULTZ, F. T., and W. E. BRILES. 1953. The adaptive value of blood group genes in chickens. Genetics *38*: 34–50.

SPRAGUE, L. M. 1957. Studies of the inheritance of certain antibodies and soluble blood group substances found in normal bovine sera. Doctoral dissertation, Univ. California.

——— 1958. On the recognition and inheritance of the soluble blood-group property "Oc" of cattle. Genetics *43*: 906–912.

STORMONT, C. 1947. Immunogenetic studies of the erythrocytes of cattle. Doctoral dissertation, Univ. Wisconsin.

——— 1947–58. Unpublished data.

——— 1949. Acquisition of the J substance by the bovine erythrocyte. Proc. Nat. Acad. Sc. U.S. *35*: 232–237.

——— 1950. Additional gene-controlled antigenic factors in the bovine erythrocyte. Genetics *35*: 76–94.

——— 1952. The F-V and Z systems of bovine blood groups. Genetics *37*: 39–48.

——— 1954. Erythrocyte mosaicism in a heifer recorded as single-born. J. Animal Sc. *13*: 94–98.

——— 1955. Linked genes, pseudoalleles and blood groups. Am. Natur. *89*: 105–116.

STORMONT, C., and R. W. CUMLEY. 1943. Cellular antigens in cattle blood. J. Hered. *34*: 35–41.

STORMONT, C., W. J. MILLER, and Y. SUZUKI. 1957. New phenogroups in the B system of bovine blood groups. Genetics *42*: 400 (Abstract).

STORMONT, C., R. D. OWEN, and M. R. IRWIN. 1951. The B and C systems of bovine blood groups. Genetics *36*: 134–161.

STORMONT, C., Y. SUZUKI, and B. A. RASMUSEN. 1957. Cross reactions of ovine and bovine isoimmune antibodies in cattle and sheep blood-typing studies. J. Animal Sc. *16*: 1102 (Abstract).

STORMONT, C., W. C. WEIR, and L. L. LANE. 1953. Erythrocyte mosaicism in a pair of sheep twins. Science *118*: 695–696.

EVIDENCE FROM TWINS ON VARIATION IN GROWTH AND PRODUCTION OF CATTLE

H. P. Donald[1]

EVER SINCE Galton's time, the astonishing resemblance of monozygotic twins has, like many another useful fact, been sometimes misused. They tempt the investigator to overestimate or underestimate the strength of heredity, but, as Hogben (1933), Price (1950), and others have been at pains to point out, monozygotic twins alone cannot unscramble the phenotype into its component parts of nature and nurture. Where these twins have failed, however, the single-born can claim no great success. In the circumstances, an attempt to reconcile the results obtained from twins and from non-twins may advance the main problem towards a solution.

It is a feature of all mammalian material that it may result from a biased sampling of both the relevant gene pool and the relevant flux of environment. Although no assessment of bias in samples of cattle seems possible at present, it is as well to consider this difficulty.

To begin with, there may be a systematic difference between eggs or zygotes that produce singles and those that undergo splitting to form monozygotic twins. During gestation, particularly in the early stages, a substantial loss of embryos of both singles and twins occurs. It seems hardly likely that this loss is random with respect to the genotypes of dam and offspring, or to uterine environment. The treatment of MZ pairs as genetically identical may lead to error. Darlington (1954) has dealt with this subject, but the effects of cytoplasmic inequalities or mutation remain unmeasured.

Occasional mistakes in diagnosing MZ and DZ twins are doubtless made. Singles may include some twins that have lost their mates through pre- or post-natal death or through separation. Early deviations from normal diploid development may occur to blur the distinction between twins and singles.

Cattle twins, of course, set up a confluence of foetal circulations. This has well-known consequences for heifers twin to bulls and for red-cell and skin antigens; but are there any others? Fraternal twins maintain their individualities over a wide range of morphological and biochemical characters, even of blood, so much so that one-egg twins can be recognized with a good deal more assurance than the 5 per cent level of probability confers. Vascular anastomosis may both provoke and prevent variation. The mixing of bloods and migration of cells could disturb normal development but, at the same time, even out nutritional inequalities.

Before their first lactations are completed, the numbers of heifers are thinned —by disease and death, by untimeliness of birth, by ill-favoured appearance, and so on. Just how representative of the original population of zygotes and of environmental histories the survivors are is a question for those who use mass data from commercial herds. Twins in these herds may not be subject to the same degree of selection. MZ twins in experimental herds certainly are not. Only a tiny fraction of those born are offered for sale and bought, but thereafter there is no

[1]A.R.C. Animal Breeding Research Organisation, Edinburgh, Scotland.

culling except that necessary because of sterility or gross ill health. Such twins provide a very different sort of sample of the cattle population, but they may nevertheless be genetically more representative than selected singles are.

Sampling animals means sampling their previous environments. What effect this sampling has on the performance of twins and singles is obscure. Twins are manifestly smaller than singles at birth and for some time thereafter. In our experience, by the time they are two years old, twins of either type are, on average, very similar to singles. In weight and dimensions they have become indistinguishable. Fertility has been equally good. Yields of milk and butterfat percentage during first lactations are also similar both in commercial herds (Ward, 1948; Watson, unpublished) and in our experimental herd. The undeniable handicap of twins at birth, therefore, seems to be overcome by degrees, at least to the point where any remaining difference is swamped by variation emerging later.

Variation in Growth

Information bearing on some of these questions may be gathered from an experiment set up by the Animal Breeding Research Organisation to compare both types of twin with paired singles of several degrees of relationship (Donald, 1953). Pairs have been bought at one to two weeks of age and reared on the same farm. As calves, they were bucket-fed on milk for twelve weeks. Animals were fed in winter according to the same scale of rations but, since the dates of birth varied between pairs, the age incidence of changes from summer grazing to winter feeding varied. For brevity, groups will be denoted as follows: MZ = one-egg twins; DZ = two-egg twins; FS = full-sibs, contemporary offspring of MZ pairs; HZ = paternal half-sibs, sires all different; U = unrelated paired individuals. The U group was entirely of the Ayrshire breed, but the others were of varied breed composition. In what follows, however, all variances, unless otherwise described, have been calculated within breed or first cross.

Except for the full-sib group, all pairs were female. This group, unlike the others, comprised home-bred animals and was kept with a view to comparing full-sibs that were twins with those that were contemporary (that is, born within two weeks of each other) but not twins. Pairs of mixed sex have had the average effect of sex removed from the variance.

From the five groups, numerous other paper groupings of unrelated and non-contemporary animals may be made up. Of these, only those derived from MZ, DZ, or U animals adjacent in the serial order of their birth dates are to be mentioned here. The four groups so obtained are detailed in Table I. Unlike the others, members of U_{xt} pairs are not matched for breeding and so contain within pairs some fraction of breed differences. Dates of birth of members of these artificial pairs may differ by a week to six months or more.

At three months of age, that is, weaning time under our régime, the intra-pair variances in liveweight were as set out in Table I.

Sampling errors attached to these estimates are large and it is therefore unwise to set much store by individual items in this table. It seems safe enough, however, to conclude that twins resemble each other more closely than related or unrelated singles; that non-contemporary individuals differ more than contemporaries; and that the inclusion of some breed variation within pairs decreases the resemblance still more.

TABLE I

INTRA-PAIR VARIANCE OF LIVEWEIGHT AT THREE MONTHS OF AGE BEFORE AND AFTER ADJUSTMENT FOR COVARIANCE WITH WEIGHT AT TWO WEEKS OF AGE

Mates	Group	Variance (lb.²)		Percentage difference	No. of pairs
		Unadjusted	Adjusted		
Contemporary					
One-egg twins	MZ	96	84	13	116
Two-egg twins	DZ	130	71	45	65
Full-sibs	FS	273	144	48	45
Half-sibs	HZ	327	100	69	33
Unrelated	U	212	108	49	27
Non-contemporary					
Ayrshire, singles	U_{as}	291	181	40	26
Ayrshire, twins	U_{at}	444	214	53	36
Combined breeds, twins	U_{bt}	404	217	46	95
Crossbreds, twins	U_{xt}	550	340	38	68

The fact that the FS pairs showed a much larger variance than the DZ pairs may be evidence that the prenatal environment of twins is more uniform than that of singles. It cannot be finally accepted as such, however, until allowance is made, not only for the larger birth-weight of the single-born FS calves and the inclusion of crossbreds among them, but also for the possibility that there has been some selection of DZ pairs for uniformity. DZ pairs with crossbred dams, however, showed practically the same variance at three months and at eighteen months as those with purebred dams. They have been combined in Tables I and II. To date, the U and U_{as} pairs have shown a relatively small variance, and they are almost certainly not fully representative of the range of birth weights of singles.

Some of these difficulties could perhaps be diminished by deducting from the intra-pair variance in three-month weight that part of it which was associated with intra-pair differences in birth weight. Since, however, birth weights were not available, liveweights at two weeks of age had to be used instead for estimating the covariance of the weights at the two ages. After allowance was made for this, the adjusted variances in three-month weight shown in Table I were obtained. It appears that about half the variance was removed by this process except for MZ pairs where only 13 per cent was removed. Their original small differences were rather transitory. The fact that all other groups showed similar percentage reductions suggests that no matter whether the differences in weight at two weeks were large or small, genetic or environmental in origin, they were connected in some way with half the variance within pairs at three months of age. The other half seems to have been mainly environmental in origin. In the first place, the adjusted variances show little increase from MZ to U pairs; and in the second, they are twice as great in non-contemporary pairs of the same breed.

Inequalities of environment are apparently not randomly distributed. Litter mates, so to speak, up to the age of three months have environments which are correlated. The C fraction of environmental variance (Lush, 1948; Lerner, 1950) is large.

By the time the animals reached eighteen months of age, heredity had become a much more potent source of variation. As Table II shows, their weights then varied increasingly as relationship diminished. Pairs made up of unrelated non-contemporary animals again showed more intra-pair variation than the HZ or U pairs,

TABLE II

INTRA-PAIR VARIANCE OF LIVEWEIGHT AT EIGHTEEN MONTHS OF AGE BEFORE AND AFTER ADJUSTMENT FOR COVARIANCE WITH LIVEWEIGHT AT THREE MONTHS OF AGE*

Mates	Group	Variance (lb.²)		Percentage difference	No. of pairs
		Unadjusted	Adjusted		
Contemporary					
One-egg twins	MZ	396	309	23	100
Two-egg twins	DZ	1,462	789	46	74
Full-sibs	FS	1,448	855	42	44
Half-sibs	HZ	2,049	1,202	43	40
Unrelated	U	1,643	1,297	24	23
Non-contemporary					
Ayrshire, singles	U_{as}	2,528	1,412	47	22
Ayrshire, twins	U_{at}	2,915	1,352	55	34
Combined breeds, twins	U_{bt}	2,499	1,652	35	88
Crossbreds, twins	U_{xt}	4,101	2,137	49	70

*Numbers of pairs differ in Tables I and II because some animals had not reached eighteen months of age and others lacked recorded weights.

especially when the individuals making up the pairs were not uniform as to breed (U_{xt}). In most groups, also, intra-pair variance in eighteen-month weight was considerably reduced when previous differences at three months of age were taken into account. The percentage reduction was in fact of the same order as that obtained when differences in three-month weight were adjusted for those at two weeks.

MZ pairs were again exceptional in this respect. The U pairs were also exceptional, but since their numbers are regrettably small the reason for this is assumed to be sampling variation.

SOURCES OF VARIATION

On the evidence of Tables I and II, at least four sources of variation must be postulated: e^2—environmental variation occurring within twin pairs; m^2—variation additional to e^2 occurring within pairs of contemporary non-twins; t^2—variation additional to e^2 and m^2 occurring within pairs of non-contemporaries; g^2—genetic variation. It is to be understood that, on present evidence, the use of m^2 and t^2 implies nothing about causes. The same causes in fact might contribute to both. In terms of the four specified components, within-pair variances can be expected to range from e^2 for MZ pairs to $e^2 + g^2 + m^2 + t^2$ for U_{as}, U_{at}, and U_{bt} pairs. Correspondingly, between-pair variances will range from $e^2 + 2g^2 + 2m^2 + 2t^2$ for MZ pairs to $e^2 + g^2 + m^2 + t^2$ for the U_{as}, U_{at}, and U_{bt} pairs. Several estimates of heritability can be extracted from such material. The highest is likely to come from the intra-class correlation of MZ pairs which is $(g^2 + m^2 + t^2) / (e^2 + g^2 + m^2 + t^2)$ and the lowest from the ratio $g^2 / (e^2 + g^2 + m^2 + t^2)$. Variance within MZ pairs is clearly not a measure of all environmental variance.

For an example of the changing importance of sources of variation, an analysis of intra-pair variances in liveweight has been carried out. For the purpose, these have been defined (King and Donald, 1955) as:

$$\begin{aligned} \text{MZ} &— e^2 \\ \text{DZ} &— e^2 + \tfrac{1}{2}g^2 \\ \text{FS} &— e^2 + \tfrac{1}{2}g^2 + m^2 \\ \text{HZ} &— e^2 + \tfrac{3}{4}g^2 + m^2 \\ \text{U} &— e^2 + g^2 + m^2 \end{aligned}$$

Solution of the equations by the least squares method gave values of e^2, g^2, and m^2 which have been converted to percentages of their sum. For comparison, a similar set of solutions ignoring m^2 has been worked out. Both sets appear in Table III.

TABLE III

SOURCES OF INTRA-PAIR VARIANCE IN LIVEWEIGHT DERIVED BY THE LEAST SQUARES METHOD AND EXPRESSED AS PERCENTAGES OF THEIR SUM

Variance at	With m^2			Without m^2	
	e^2	g^2	m^2	e^2	g^2
2 weeks	29	21	50	36	64
3 months	36	18	46	42	58
3 months (adjusted)	62	—	38	86	14
18 months	18	82	—	18	82
18 months (adjusted)	23	72	5	23	77

The outcome of fitting all three gives an intelligible result conforming to expectation. At or near birth, the dam and other factors associated with twinship are important (m^2). In time, they become negligible, although for other reasons contemporaries, as distinct from twins only, may continue to have environments common to some extent. About weaning time, environmental variation reaches its maximum. By the time the animals were one and a half years old, heredity had asserted itself until it accounted for about 75 per cent of all variation. When m^2 is not fitted, g^2 assumes at the younger ages much higher values.

More complicated equations for partitioning variance can be set up which include components for dominance and epistatic effects (Kempthorne, 1955; Le Roy, 1957). With the numbers of pairs at present available, however, it seems that their solution would turn out to be a somewhat inconclusive exercise. Those types of genotype-environment interaction possible within the limits of a uniformity trial doubtless contribute to the total variance, but so far no attempt has been made to estimate deviations from an additive scheme due to them. Observation of one-egg twins suggests, however, that such interactions may be collectively important but individually erratic and infrequent. Teat shape, for instance, a highly heritable character, can deviate so much on occasion from the norm as to make milking difficult or painful and thus depress yield. With a specially adapted machine, the resulting yield would be quite different. Temperamental deviations that could produce interactions of the same type have been observed.

Some fraction of this sort of variance will appear as additively genetic, more so perhaps in a twin experiment where efforts are made to keep treatment constant than in commercial practice where management may be varied to meet peculiarities of the individual.

COMPARISON OF HERITABILITIES

The treatment of such non-additive elements as genetic has been suggested as one reason why estimates of heritability equivalent to the intra-class correlation of MZ twins are regularly very high (for example, Patchell, 1956). In the light of the foregoing evidence on body weight, a more likely reason is that the $(m^2 + t^2)$ portion of the variance is, in effect, included with the genetic portion. It may not, however, be true for all characters or all conditions. Where $(m^2 + t^2)$ is very small, the correlation becomes $g^2 / (e^2 + g^2) = h^2$ as estimated from the intra-pair variances of MZ and DZ pairs. It seems worthwhile, therefore, to set up a com-

parison of heritabilities obtained from various sources. They fall into four categories: (*a*) intra-class correlation of MZ twins; (*b*) intra-pair variances of MZ and DZ twins; (*c*) data from progeny-testing stations; (*d*) field data, mostly parent-offspring or half-sib correlations. In no category are all available estimates quoted, but a representative selection where they are numerous is given in Table IV. The twin data shown are all original, but no further account is taken here of the FS, HZ, and U groups. For data on milk and its constituents, I am indebted to Dr. J. H. Watson, and for those on body measurements to Dr. St. C. S. Taylor. Previous work on twins has been summarized by Bonnier and Hansson (1948), Hancock (1954), and Brumby (1958), and will not be reviewed again here. The evidence for zero heritability of fertility is that MZ and DZ twins combined showed a slightly larger intra-pair variance (244 pairs, first and second pregnancies) in number of services a conception than did singles (97 pairs). Both groups showed a small intra-class correlation (0.163). This agrees with earlier work on single-born cows (Legates, 1954).

Estimates from the MZ/DZ comparison of intra-pair variances tend to be lower than those from MZ pairs alone. The difference is most marked for juvenile weights and late maturing body dimensions. It is also marked for juvenile body measure-

TABLE IV

HERITABILITIES AS PERCENTAGE FROM TWINS, PROGENY GROUPS IN TESTING STATIONS, AND FIELD DATA FROM CATTLE IN COMMERCIAL HERDS

	Twins					
	No. of pairs					
	MZ	DZ	MZ	MZ/DZ	Test station	Field
Liveweight						
2 weeks	116	65	91	50		
3 months	116	65	85	41		23 (1)
6 months	60	60	96	39		64 (2)
12 months	60	60	97	55		65 (2)
18 months	100	74	96	84	84 (1)	
Body measurements at two years						
Head width	60	60	93	91		
Head length	60	60	90	81		
Height, withers	60	60	94	86	51 (14)	73 (3); 63(13)
Width, hooks	60	60	94	80		50(13);
Width, fore-rib	60	60	86	71		
Body length	60	60	80	56		58(3)
Milk–Lact. 1						
Yield: 305-day	34	33	90	86	58(8); 75(9); 57(14)	43(4); 30(5); 25(6)
70-day	34	33	89	83		36(4); 35(7)
Fat (%)	34	33	90	82	81(8); 71(9); 84(14)	60(5); 43(4); 32(6)
Lactose (%)	34	33	88	62		36(6)
Crude protein (%)	34	33	87	66		48(6)
Rate of flow	25	23	91	90		
Fertility	—	—	16	0	0(10)	0(11); 0(12)

(1) Shelby *et al.* 1955
(2) Buiatti, 1954
(3) Touchberry, 1951
(4) Rendel *et al.* 1957
(5) Johansson, 1950
(6) Robertson *et al.* 1956
(7) Madden *et al.* 1955
(8) Johansson, 1954
(9) Robertson and Mason, 1956
(10) Rottensten and Touchberry, 1957
(11) Dunbar and Henderson, 1953
(12) Legates, 1954
(13) Weber, 1957
(14) Mason *et al.* 1957

ments, but these are not shown in Table IV. For early maturing characters, such as head width, for milk yield, fat percentage, and rate of flow—an anatomically determined trait—it is very slight (cf. Brumby and Hancock, 1956). Differences of an intermediate size are shown by lactose percentage, crude protein percentage, and fertility.

Information from testing stations is limited to that coming from Denmark for Red Danish dairy cattle, and from Montana R.O.P. trials with Hereford beef cattle. Heritabilities from these sources agree closely with the MZ/DZ estimates for liveweight, fat percentage, and fertility, but are somewhat lower for height at withers and milk yield. Since field data yield varying estimates of heritability, it is far from certain that the values entered in Table IV are truly representative of the real position. As they stand, however, they record relatively low values of h^2, particularly for milk yield and calf weight.

In an attempt to co-ordinate the available data, Figure 1 has been constructed by plotting heritabilities from all sources against heritabilities from field data. Only a single value for each character is shown and, if clarity is thereby improved, that value may accord only approximately with published values. The resulting idealistic diagram shows that three methods of estimation agree on a value of zero for fertility. MZ twins, however, show a small correlation which could be reasonably explained by a corresponding correlation of circumstances at mating time.

Weights and measurements of calves show the greatest discrepancies. For an interpretation of this, it can be supposed that m^2 and t^2 are both large and both contribute to the close resemblance of MZ twins. At the other extreme, h^2 from field data is low because both are distributed within sib or parent-offspring groups. Occupying an intermediate position is the h^2 from MZ/DZ comparison, and it does so by eliminating m^2 and t^2. Which of the three estimates would be most useful would depend on the purpose it is to serve.

Milk yield shows the next largest discrepancies. For this character, the two twin methods give similar results which indicates that e^2 is small relative to g^2 in spite of the possibility that some part of t^2 has become distributed within as well as between sets of twins because, owing to differences in calving date, members of twin pairs are no longer very close contemporaries. This could happen through control of environment, particularly housing and nutrition, and low culling rates. The same reasons could explain the intermediate values from test station data. These are lower than for twins because of the inclusion of some part of t^2 within sib groups, but higher than for field data because of uniformity of environment.

When h^2 from field data is moderately high as it is for fat percentage and mature body measurements reflecting bone growth, there is less room for divergent estimates. At these levels, the narrower differences observed among methods of estimation suggest that m^2 and t^2 are of diminished importance. Twins, however, having been subject to similar environments for their whole lives continue to show somewhat higher values. Heifers lactating in test stations have been together only for the latter part of their lives and heifers in commercial herds for less still on average. Another factor which may be significant for these characters is accuracy of measurement. Fat determinations made monthly produce lactation averages more subject to sampling and testing variation than the observations made weekly or even oftener on test station or experimental cattle. When monthly fat tests were used for the twins, the heritability was 10 per cent lower. Increased accuracy is achieved for a different reason with body measurements. Cattle that have been handled frequently from birth onwards have no fear of calipers or the act of measurement and there-

fore stand far more quietly and naturally than those which have not. A good deal of variation in h^2 from field data can be expected for this reason as well as for others such as the condition of the animals and the degree of maturity of the characters selected for measurement. Head width, for instance, at two years of age is much nearer its maximum than say body length. All such characters, and body weight as well, sum up previous nutritional and health history in a very different way from that shown by milk yield. Whereas the power of recovery from a check enables an animal to eventually develop its inherent conformation, there is neither the time nor the resilience necessary to allow this for milk yield which is highly dependent on current environment.

The rather limited information about h^2 for crude protein percentage and lactose percentage does not fit very well into the relationships of Figure 1. In view of the known low variability of milk constituents relative to that of milk yield, the results from MZ twins alone suggest that field data should give an h^2 of about 60 per cent as for fat per cent. From the MZ/DZ comparison, however, h^2 is only about 65 per cent. The corresponding value for field data from Figure 1 would then be 25 per cent instead of the 36 per cent for crude protein and 48 per cent for lactose found by Robertson *et al.* (1956). These authors, however, found an h^2 of only 32 per cent for fat content. Further data will be necessary to determine where the inconsistency arises.

Discussion

No attention has been paid to gene $\times$ gene or gene $\times$ environment interactions, or to differential culling as causes for the variation in estimates of h^2 because there is no evidence concerning them. There would seem, however, to be little need to call on them when the observed differences are small and easily accounted for in more obvious ways. Since the widest differences occur in the estimates of h^2 for milk yield and juvenile characters, the greatest scope for interactions would appear to lie in them.

As it is to be expected, all sources of information agree in a general way about which characters lie at the extremes of the scale of heritability—fertility at one end, and red-cell antigens, colour, and horns at the other. When the intermediates are fitted in, it is apparent that those which are most dependent on current environment are grouped together at the low end, and those which are least dependent at the high end. The former are also most subject to inbreeding depression. Loss of heterozygosity means a sensitivity to environment that is brought out clearly by the different results from twins and non-twins.

If the relationships shown in Figure 1 are somewhere near the truth, they can be put to practical use in several ways. First, they can assist in tracing the causes of disagreement in estimates of heritability from field data. Secondly, observations not normally made on commercial cattle can be carried out on twins in experimental herds and form the basis for deductions about the results likely to be obtained from singles in the field. For this purpose, it will be advantageous to be able to classify a given character into one of several broad groupings:

Heritability

Very high—colour, horns, red cell antigens
High—mature anatomical features
Moderately high—milk constituents
Moderately low—milk yield
Low—calf weights and measurements
Very low—fertility

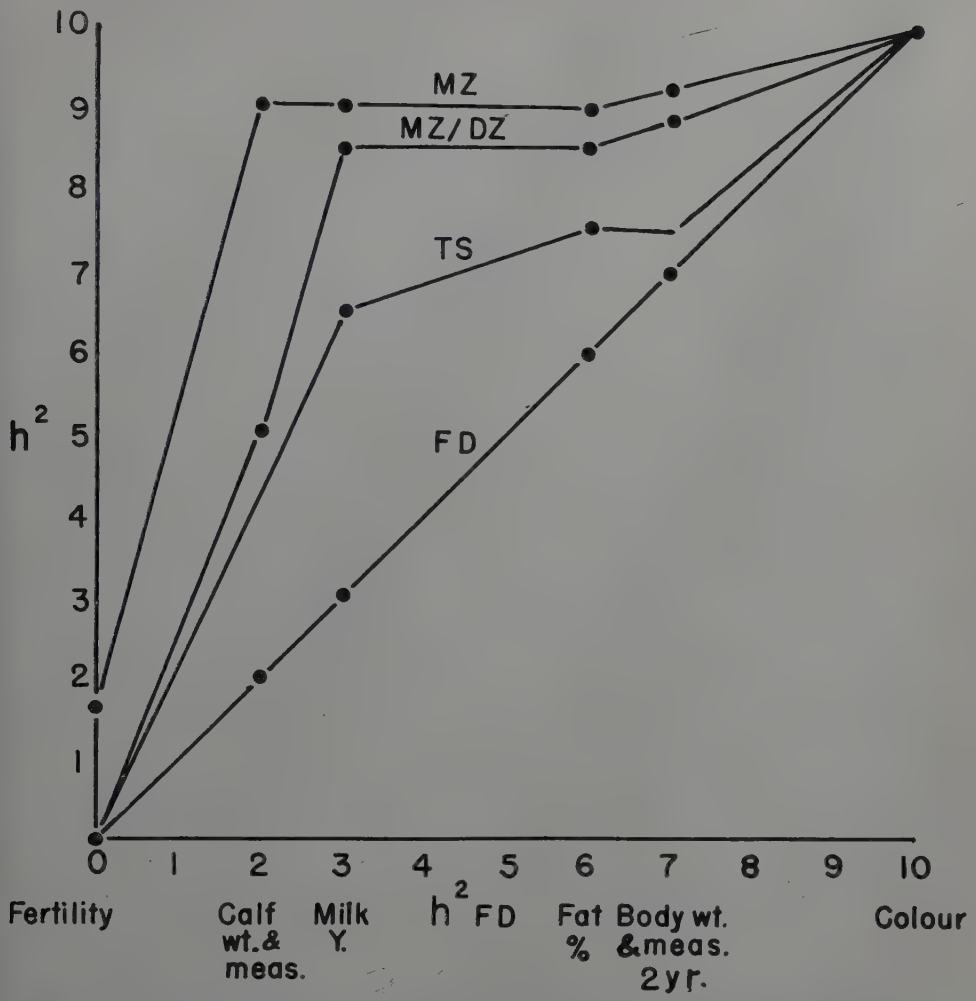


FIGURE 1. Heritabilities from twins and non-twins plotted against selected heritabilities from field data. TS, test station; FD, field data.

An h^2 from a sample of MZ twins of, say, 0.9 could, without further specification, correspond to an h^2 in the field varying from 0.2 to 0.7. If, however, body length of a young calf was of interest, the value of 0.2 would be appropriate; but 0.6 if the character was a milk constituent, and 0.7 if a mature anatomical feature. Maximum rate of milk flow for instance, which shows an h^2 of 90 per cent in twins, should turn out to have an h^2 of about 70 per cent in commercial cattle. Yield of milk during the first seventy days of lactation gave an h^2 of about 85 per cent in twins and may be expected to fall, like 305-day lactation itself, into the 20–40 per cent range. Rendel *et al.* (1957) have in fact reported an h^2 of 36 per cent in first lactations.

Thirdly, there are implications for non-genetic uses of twins in, for example, nutrition experiments. Twins are expedient where random hereditary variation is undesirable—as has long ago been pointed out. This advantage loses its force where such variation is weak, but this loss may be more than made good by the oppor-

tunity twins create of segregating a large part of the environmental variance into the $(m^2 + t^2)$ fraction effective only between pairs. Trials involving calves or milking cows would profit most from this advantage. Under some circumstances, application of the principle in respect of t^2 may be possible in the assessment of dairy bulls if their daughters can be compared within limited calving seasons as in New Zealand or in testing stations.

Summary

Variances in liveweight at three months and eighteen months of age are reported for paired animals of differing degrees of relationship ranging from 100 per cent (one-egg twins) to 0 per cent (unrelated). In five types of pair, the two members are contemporary—that is, born within two weeks of each other—and reared together under uniform management. In four more types of pair, obtained by reassorting the same animals, the unrelated members are non-contemporary.

At both ages, intra-pair variance increased as relationship diminished but more so at eighteen months. It also increased substantially from contemporaries to non-contemporaries. Except for MZ pairs, about half the variance was associated with differences at an earlier age.

Four sources of variation can be recognized: e^2, within MZ pairs; m^2, additional for non-twin contemporaries; t^2, additional for non-contemporaries; and g^2, genetic. The proportions of the total accounted for by each source vary with age and character.

Against this background a comparison is made of heritabilities obtained from MZ twins alone, MZ and DZ twins in combination, testing stations, and commercial herds. The differences are greatest for weights and measurements of calves and milk yield, and least for fertility, fat percentage, and mature measurements. They are interpreted in terms of the changing importance of the sources of variation, control of environment, and observational error.

REFERENCES

Bonnier, G., and A. Hansson. 1948. Identical twin genetics in cattle. Heredity *2*: 1–27.

Brumby, P. J. 1958. Monozygotic twins and dairy cattle improvement. Animal Breed. Abstr. *26*: 1–12.

Brumby, P. J., and J. Hancock. 1956. A preliminary report of growth and milk production in identical- and fraternal-twin dairy cattle. N. Zealand J. Sc. *38* (A.): 184-193.

Buiatti, P. G. 1954. Ereditabilità e correlazione fra peso vivo e altezza al garrese in vitelle di razza chianina. Riv. Zootec. *27*: 59–63.

Darlington, C. D. 1954. Heredity and environment. Proc. IX Internat. Congr. Genet. *1*: 370–381.

Donald, H. P. 1953. A study of variation in twin cattle. I. General description. J. Dairy Res. *20*: 355–360.

Dunbar, R. S., Jr., and C. R. Henderson. 1953. Heritability of fertility in dairy cattle. J. Dairy Sc. *36*: 1063–1071.

Hancock, J. 1954. Monozygotic twins in cattle. Advance. Genet. *6*: 141–181.

Hogben, L. 1933. Nature and Nurture. London: George Allen and Unwin. Pp. 144.

Johansson, I. 1950. The heritability of milk and butterfat yield. Animal Breed. Abstr. *18*: 1–12.

——— 1954. An analysis of data from the Danish bull progeny testing stations. Zsch. Tierzücht. Züchtungsbiol. *63*: 105–126.

Kempthorne, O. 1955. The theoretical values of correlations between relatives in random mating populations. Genetics *40*: 153–167.

KING, J. W. B., and H. P. DONALD. 1955. A study of variation in twin cattle. III. Growth. J. Dairy Res. *22*: 1–9.

LEGATES, J. E. 1954. Genetic variation in services per conception and calving interval in dairy cattle. J. Animal Sc. *13*: 81–88.

LERNER, I. M. 1950. Population Genetics and Animal Improvement as Illustrated by the Inheritance of Egg Production. Cambridge: University Press.

LE ROY, H. L. 1957. Methods of determining the heritability of characters in investigations with twins. Zsch. menschl. Vererb. *34*: 145–170.

LUSH, J. L. 1948. Heritability of quantitative characters in farm animals. Proc. VIII Internat. Congr. Genet., pp. 356–375.

MADDEN, D. E., J. L. LUSH, and L. D. MCGILLIARD. 1955. Relations between parts of lactations and producing ability of Holstein cows. J. Dairy Sc. *38*: 1264–1271.

MASON, I. L., ALAN ROBERTSON, and B. GJELSTAD. 1957. The genetic connexion between body size, milk production and efficiency in dairy cattle. J. Dairy Res. *24*: 135–143.

PATCHELL, M. R. 1956. A uniformity trial with monozygous twin cows. N. Zealand J. Sc. *38*(A): 1–14.

PRICE, B. 1950. Primary biases in twin studies: A review of prenatal and natal difference-producing factors in monozyogtic pairs. Am. J. Human Genet. *2*: 293–352.

RENDEL, J. M.; ALAN ROBERTSON; A. A. ASKER; S. S. KHISHIN; and M. T. RAGAB. 1957. The inheritance of milk production characteristics. J. Agr. Sc. *48*: 426–432.

ROBERTSON, ALAN, and I. L. MASON. 1956. The progeny testing of dairy bulls: A comparison of special station and field results. J. Agr. Sc. *47*: 376–381.

ROBERTSON, ALAN, R. WAITE, and J. C. D. WHITE. 1956. Variations in the chemical composition of milk with particular reference to the solids-not-fat. J. Dairy Res. *23*: 82–91.

ROTTENSTEN, K., and R. W. TOUCHBERRY. 1957. Observations on the degree of expression of estrus in cattle. J. Dairy Sc. *40*: 1457–1465.

SHELBY, C. E., R. T. CLARK, and R. R. WOODWARD. 1955. The heritability of some economic characteristics of beef cattle. J. Animal Sc. *14*: 372–385.

TOUCHBERRY, R. W. 1951. Genetic correlations between five body measurements, weight, type and production in the same individual among Holstein cows. J. Dairy Sc. *34*: 242–255.

WARD, A. H. 1948. Wastage among twins and their production as two-year-olds. N. Zealand Dairy Board 24th Annual Rep., p. 48.

WEBER, F. 1957. Die statistischen und genetischen Grundlagen von Körpermessungen am Rind. Zschr. Tierzücht. Züchtungsbiol. *69*: 225–260.

THE GENETICS OF MILK YIELD

Ivar Johansson[1]

In the first decades after the rediscovery of Mendel's laws of heredity, studies of the causes of variation in milk yield used the same approach as that used in studies of qualitative characters. More or less complicated genetic formulas were devised, based on field data, to explain the variation between animals, ranging from Professor James Wilson's one-factor hypothesis of 1910 to Freiherr von Patow's more elaborate scheme of 1926. In the meantime several cross-breeding experiments were started with the hope that it would be possible to discover, under controlled environmental conditions, *how* the capacity for milk yield was inherited. All these efforts failed owing to the complexity of the problem.

The capacity of milk yield depends on numerous physiological processes, and most of these are probably influenced by many genes. Furthermore, a multitude of environmental factors influences the manifestation of the character which is measured quantitatively. It has been said that for each cow there is a ceiling in regard to the quantity of yield and that it is impossible to raise the amount of yield above this by any improvement in feeding and management. If we think of a ceiling with the form of a cupola, no objection can be made. However, if the ceiling is supposed to be at right angles to the walls, the expression is misleading, because we have to take into account a diminishing effect of several of the environmental factors when they are increased above a certain level. The actual yield of a cow is simply the manifestation of her genotype under a given set of environmental conditions. Under another set of conditions the yield may be more or less. The absolute maximum yield which may be obtained under optimum conditions is very seldom reached.

The advance of population genetics made a new approach possible. Statistical methods offered a means of estimating the relative importance of distinguishable sources of variation in quantitative characters. From the application of these methods the concept of *heritability* emerged, now widely used in relation to animal-breeding problems.

I am going to discuss not the methods of statistical analysis but the interpretation of some of the results which have been obtained in such analyses. When not otherwise stated, I refer to heritability in the "narrow meaning" of the term (J. L. Lush), that is, the fraction of the total or phenotypic variance which is caused by the average or additive effect of the genes in the population studied. Hitherto there has been very little evidence for any marked effect of dominance or epistasis when the deviations from random mating were comparatively small. It has been shown that the heritability of the total milk yield during the lactation period is approximately the same as that of the amount of butterfat in the same period, and therefore these two measures of the quantity of yield will be used interchangeably.

It may seem obvious that, since the coefficient of heritability is the ratio between the variance caused by the additive effect of the genes and the total variance,

[1]Department of Animal Breeding and Genetics, The Agricultural College of Sweden, Uppsala.

different values may be obtained under different conditions. Generally, differences in this ratio (h^2) have been attributed to differences in the denominator due to environmental variations and errors in measurement, or to the effect of selection and the mating system on the numerator. However, an estimate of the genetic variance of a highly modifiable character may turn out to be rather different under different environmental conditions because the same genotypes will manifest themselves more or less clearly in different environments.

Some years ago we found in an analysis of field data from the Swedish Red and White cattle a significantly higher dam-daughter correlation for the butterfat yield in the first than in the second lactation (Johansson and Hansson, 1940). Later this result was verified on another set of data from the same breed and also for the Swedish Friesians (Johansson, 1955), as well as by Rendel *et al.*, (1957) on data for six British breeds (Table I). The yield during the first lactation is influenced by the age and the nutritional state of the cow at calving, but there is no preceding dry period to enter in as a variable. The second lactation yield is much more sensitive to variations in length of the calving interval or dry period than are the later lactations, and probably it is also more sensitive to environmental influences. This sensitivity was shown by Sanders (1927) and has been verified in our investigations. I would suppose that this is the main explanation of the difference in heritability of the first and second lactation yields.

TABLE I

HERITABILITY OF MILK YIELD IN DIFFERENT LACTATIONS AND ON DIFFERENT LEVELS OF AVERAGE HERD YIELD

Order of lactation	Johansson, 1955	Rendel *et al.*, 1957
First	0.33±0.06	0.43±0.06
Second	0.10±0.05	0.24±0.07
Third	0.24±0.04	—
Level of herd average yield	**Mason and Robertson, 1956**	**Gravert, 1958**
Low	0.05±0.04	0.13±0.05
Medium	0.12±0.04	0.21±0.06
High	0.22±0.05	0.30±0.07

Mason and Robertson (1956) analyzed field data from the Danish Cow Testing Associations and found that the variance among sires increased more than the variance within progeny groups with a rising average herd yield when three levels of yield were compared: low (3,447 kg. milk on the average), medium, (3,917 kg.) and high (4,391 kg.). The difference between the high and low planes in the heritability estimates were significant (Table I). Gravert (1958) studied the regression of daughters as compared to their dams ("within sires") using the first record for each animal expressed as a percentage of the simultaneous herd average, and when he divided the herds into three groups according to their average yield, he found the same trend in the heritability estimates that Mason and Robertson did. However, Legates (1957), in a similar analysis of American Jersey records, found about the same heritability at different production levels. With Swedish data we have found somewhat lower values in low- than in high-producing herds, but the difference was not significant (Johansson, 1953).

Although the results are somewhat divergent, they indicate, nevertheless, that higher estimates of genetic variance and heritability may be obtained on a high level of feeding and management.

In a survey of the literature on estimates of the heritability of milk yield it seems appropriate to divide the reports into three groups depending on whether the data originate (i) from the field without any particular control of the environmental conditions, (ii) from special stations or experimental herds where such control has been exercised, or (iii) from identical twin research. In Table II results are presented from these three sources.

TABLE II

HERITABILITY OF MILK AND BUTTERFAT YIELD ESTIMATED FROM DIFFERENT TYPES OF DATA

	Repeatability		Heritability		
	Milk or butterfat	Fat percentage	Milk or butterfat	Fat percentage	Author
Field data*					
Various dairy breeds (U.S.A.)	—	—	0.25	0.50	Lush and Schultz, 1936
Ayrshires (U.S.A.)	—	—	0.31	0.55	Tyler and Hyatt, 1947
Jerseys (U.S.A.)	0.41	—	0.20	—	Legates and Lush, 1954
6 dairy breeds (Great Britain)	0.48	0.55	0.43	0.43	Rendel *et al.*, 1957
Ayrshires (Great Britain)	0.46	0.69	0.31	0.54	Mahadevan, 1951
Red and White cattle (Sweden)	0.43	0.64	0.39	0.68	Johansson, 1953
Friesians (Holland)	—	—	0.35	0.76	Fattah, El-Shimy, 1957
Danish progeny-testing stations†	—	—	0.58	0.81	Johansson, 1954
Identical twin studies					
Ruakura					
10 twin pairs in uniform E‡	—	—	0.86	0.96	Hancock, 1953
15 twin pairs on diff. E level‖	—	—	0.90	0.95	Hancock, 1953
Wiad					
14 twin pairs on diff. E level‖	—	—	0.75	0.90	Johansson and Claesson, 1957

*h^2 calculated from the regression of daughters on dams within sires.
†h^2 calculated from variance between progeny groups within years and stations.
‡E = environment.
‖Corrections made for differences in environmental level between twins of the same pair.

When the heritability of milk yield is estimated from the regression of daughters on dams "within sires" on the basis of field data, the value of h^2 usually varies between 0.2 and 0.4, with an average for different estimates close to 0.3. The repeatability is not very much higher, on an average 0.4, indicating that variance from non-additive sources is of comparatively little importance. Higher correlation is obtained between consecutive yields than between lactations further apart. The heritability estimates for butterfat percentage are considerably higher, varying between 0.4 and 0.8, with an average of about 0.6.

In an analysis of data from the Danish progeny-testing stations, results have been obtained which differ markedly from the field results. Basing my estimates on the variances between sire-progeny groups tested simultaneously at the same station, I have found the following values for heritability in regard to 250-day records: milk

yield, 0.58; butterfat yield, 0.54; and butterfat percentage, 0.81 (Johansson, 1954). It was found, however, that significant differences existed between progeny groups, tested at the same station and in the same year, in regard to age at calving, month of calving, and length of service period, and there were indications also of differences in the condition of the heifers on arrival at the station. Furthermore, Robertson and Mason (1956), who analysed the same data, suggest that the fact that the heifers are kept in progeny groups in the barn, instead of being randomized, may cause environmental differences between the groups. That the variation in yield between progeny groups is not entirely genetic in origin seems to be quite certain; nevertheless, I am inclined to conclude from my studies of the Danish progeny-testing data that the non-genetic differences between groups are responsible for only a part of the difference in heritability as estimated from the station and field data, or in other words that the genetic variation between individuals and progeny groups is more clearly manifested under the station conditions than in the field.

The estimates of heritability which have been based on identical twin research are still higher, 0.75 to 0.90 for milk yield and 0.90 to 0.95 for butterfat percentage (Table II).

The discrepancy between the results from investigations on identical twins and on field data has caused much surprise. Hancock (1953) and others have discussed the causes of this discrepancy and also mentioned the Danish progeny-testing stations as a bridge over the gap. The following factors may contribute to the difference between the twin and the field results.

(1) The heritability estimated from the identical twin data includes the effect of dominance and epistasis, and it corresponds, therefore, to heritability in the broad sense. It includes also the effect of interaction, if any, between genotype and environment. It has been shown that such interactions may be quite important in regard to some factors of management, for example, the length of milking intervals, although level of nutrition seems to be rather insignificant in this respect.

(2) On field data the heritability is calculated within herds in order to eliminate the environmental correlation between relatives, and therefore the genetic variation between the individuals will probably be less than the corresponding variation between twin pairs which are derived from various herds, even from those where the yield is not recorded.

(3) Maternal influence during the fetal period may contribute to the similarity of twins, but this factor is probably of little importance in relation to such characters as milk yield.

(4) The field data are more or less subjected to selection not only in regard to the dams but also within the daughter groups. Many daughters are culled before they have completed 305 days of lactation. In Sweden this culling averages about 12 per cent of the total number of heifers calving.

(5) The environment in which the twin heifers are raised and in which they produce milk after calving varies more between pairs than within pairs because the members of a pair pass through the same stages of growth and lactation at approximately the same time, whereas there is a rather wide variation between pairs, for example, in regard to the month of calving. When the heritability is estimated from field data and on the basis of daughter-dam regression, there will always be at least three to four years' interval between the dam and her daughter and much variation in the season of calving. Therefore, the environmental differences between dams and daughters will be much greater than between twins of the same pair.

(6) At a research station, for example, at Wiad, the heifers are fed individually according to their growth rate, and the cows according to their capacity of yield. Therefore, it may be expected that the genetic differences will be more clearly manifested at the station than they are under ordinary field conditions where the feeding can never be so carefully adjusted. Similar control of feeding at the Danish progeny testing stations produces similar results.

In my opinion this controlled feeding of heifers and cows is the major factor in raising the heritability figures for the twin and the progeny-testing station data above those from the field data.

(7) In the field, where the yield may be tested only once a month, the errors in yield measurement are much higher than they are at research and testing stations, where the production may be controlled every day—at the Danish testing stations, four days per week. The fraction of the total variance in yield which is caused by these errors of measurement is not very great, on an average probably about 2 per cent, but it does represent a contributory cause of non-genetic variation.

The figures presented in Table II for the three types of data demonstrate clearly, I think, that a heritability coefficient or the genetic variance can be applied only under the same, or at least approximately the same, environmental conditions as those prevailing where the character was measured and the estimates made. One estimate may be as reliable as the other, provided we apply it under unaltered conditions, but otherwise it may be quite misleading. The heritability figures hitherto calculated from twin research cannot be applied to field data for the estimation of breeding values of animals or the effect of selection.

In the period 1925–45 many investigations were made on the possibility of correcting for non-genetic causes of variation in the actual yield, for example, the age of the cow, season of calving, length of the calving interval and dry period, the interval between milkings, and environmental differences between herds. Knowledge of the effect of these various factors is, of course, very valuable but to try to correct for them all would seem to be futile because of their intercorrelations and interactions. It may be appropriate to correct for one factor, or a few factors, but if we correct for many we run the risk of getting rather far from the truth, at least in the individual cases. A few examples may be briefly discussed.

Up to the third or fourth calving the capacity of yield depends not only on the age of the cow but also on the ordinal number of the lactation. A first calver does not reach her maximum yield even if she is six or seven years old, apparently because more than one pregnancy is needed for the full development of the mammary gland. Furthermore, the rise in yield with increasing age is different on different planes of nutrition. The influence of the season of calving may be very different on different farms depending on the conditions of feeding and management.

The length of the preceding and current calving intervals, as well as of the dry period, influences the lactation yield. The calving interval has a very low heritability whereas the length of the dry period is about as heritable as the lactation yield. Therefore, it is a much more difficult task to correct for the effect of variations in length of the dry period than the calving interval. If corrections for length of the dry period, or the length of lactation, are based on population averages, they will tend to eliminate part of the genetic difference in yield of the animals. For cows with a genetic disposition for short dry periods, the optimum length of the calving interval is greater than for cows inclined to long dry periods.

The depressing effect on yield of a lengthening of the interval between milkings is, according to recent research on identical twins, much less than has been assumed

before. At the Wiad station, Claesson (1959) and co-workers have tested the effect of unequal intervals between the milkings when the cows are milked twice a day as well as the effect of milking only once a day. When the night interval was sixteen hours and the day interval eight hours, the decrease in milk was only 3.9 per cent compared to two milkings per day with equal intervals. When one of the twins in a pair was milked only once a day, the decrease in yield was, however, about 45 per cent compared to the yield of the other twin milked twice daily at intervals of fifteen and a half and eight and a half hours.

An interesting observation in the Wiad trials was that some cows showed a much more pronounced reaction for a widening of the milking interval than did others. These individual differences were particularly marked when the interval was increased to twenty-four hours. For eight pairs of identical twins and one set of triplets the reduction in yield varied from 14 to 85 per cent. Here the interaction between genotype and milking interval was very pronounced, suggesting that it might be possible to breed cows which show only a moderate decrease in yield when they are milked once a day instead of twice. It should be mentioned also that although the absolute decrease in yield was greater for high yielding than for low yielding twin pairs, the relative decrease, expressed as a percentage of the yield of the twin milked twice daily, was smaller. The milking interval had only a very slight effect on the fat and protein content of the milk. This is another demonstration of the fact that the composition of the milk is rather stable under varying environmental influences compared to the quantity of yield which is highly modifiable.

When the heritability estimates are made within herds and periods we eliminate not only the environmental but at the same time the genetic differences between herds and between animals producing during the various periods. When comparing production figures for individuals or groups belonging to different herds, we cannot avoid the difficulty of estimating in one way or another the heritability of the variations between herds. It is of interest to know how high this heritability is, on an average, within the population under consideration. The problem has been studied particularly by Robertson and co-workers (1955) and figures between 0.1 and 0.2 have been suggested. In the Institute of Animal Breeding at the Agricultural College of Sweden we are attempting to elucidate the same problem in two different ways:

(i) Pairs of identical twins are collected, and at about one month of age each pair is split between two different environments, for example, one member being raised in a high producing herd and the other member in a medium or a low producing herd. This project was started about three years ago, and it will take at least three more years before any results can be presented. A similar project is carried on at the Ruakura Station, New Zealand, and there some results are available which tend to show that the genetic differences between herds may be rather insignificant (Brumby, 1958).

(ii) We have studied the yield of the heifers at the Danish progeny-testing stations in relation to the average yield of the herds where these heifers have been raised. The regression of the station yield on the corresponding herd averages was found to be curvilinear. When the herds were divided into two groups, one with average yields between 110 and 170 kg. butterfat per record year and the other with averages between 170 and 290 kg., it was found that the station heifers from the low producing herds increased 0.44 kg. butterfat for each kg. increase in the average yield of their herds whereas for heifers from high producing herds (>170 kg. butterfat) the regression was zero. The body weight of the station heifers after the first calving showed a rectilinear increase of 0.47 kg. per kg. increase in the

average yield of their herds. The major part of this weight regression is probably non-genetic, being due to more liberal feeding of the heifers in the high than in the low producing herds. I am inclined to conclude that the increase in station yield with increasing yield of the herds, up to an average of 170 kg., was due mainly to improved condition of the heifers at the time of calving. Artificial insemination had been used in the herds during several cow generations and the genetic differences between these herds had therefore probably been reduced. It might be expected that the herd differences are greater where natural breeding is used and the bulls are used in one herd only. However, the genetic differences between the herds within a breed, which is not split up into separate breeding groups, are probably much smaller, on an average, than has been generally supposed. Further investigations are needed in regard to the genetic variance between herds under different systems of breeding and management.

Summary and Conclusions

By partitioning the variance of a quantitative character, for example, the milk yield, on its various sources, valuable information may be obtained as to the relative importance of these sources in a certain population and under a given set of environmental conditions. It is of interest to know not only the heritability of the character but also the fractions of the total variance which are caused by various non-genetic factors, for example, the variation in age of the cows, and the season of calving. The fractions (or percentages) obtained may show a considerable variation from one investigation to another depending on how the character is measured and what the environmental conditions and genetic structure of the population are. For example, when the fraction of the total variance caused by the varying ages of the cows was estimated on data for the first four lactations of Swedish Red and White cows, this fraction was found to be 25 per cent for the milk yield during the first two hundred days after calving, 10 per cent for the yield in three hundred days and only 3.5 per cent for the yield per record year (Johansson and Hansson, 1940). To interpret the ratios it is necessary, therefore, to know the conditions under which they were obtained.

The same is true of the heritability coefficients. The estimates of heritability may be quite different for groups within the same population if the animals of the groups are raised and have produced under different environmental conditions. This is due not only to differences in the environmental variance but also to the fact that genetic differences between individuals will manifest themselves more clearly in one environment than in another and that, therefore, our estimates of the genetic variance will be different. This would seem to be at least a part of the explanation of the great difference between heritability estimates for milk yield on the basis of field data (0.2–0.4), the Danish progeny-testing stations (about 0.6), and from identical-twin research (0.8–0.9). I suppose that the same interpretation applies also to other quantitative characters which are strongly influenced by the environment. When this is the case, we are not much better off if we limit the statistical operations to an estimate of the genetic variance rather than take the next step also and calculate the ratio between this variance and the total, that is, the heritability.

It is important that we find the right interpretations of the results obtained in the statistical analyses of the data. Here each fraction of the total variance presents its own problems, which should be carefully studied.

REFERENCES

BRUMBY, P. J. 1958. Monozygotic twins and dairy cattle improvement. Animal Breed. Abstr. *26*: 1–12.

CLAESSON, O., A. HANSSON, N. GUSTAVSSON, and E. BRANNANG. 1959. Studies on monozygous cattle twins. XVII. Once-a-day milking compared with twice-a-day milking. Acta agr. scand. *9*: (in press).

FATTAH, EL-SHIMY, S. A. 1957. The heritability of milk yield and fat percentage in the Friesian cattle in the province of Friesland. Zschr. Tierzücht. Züchtungsbiol. *69*: 321–350.

GRAVERT, H. O. 1958. Untersuchungen über die Heritabilität der Butterfettleistung. Zschr. Tierzücht. Züchtungsbiol. *71*: 155–163.

HANCOCK, J. 1953a. Studies in monozygotic cattle twins. VII. The relative importance of inheritance and environment in the production of dairy cattle. N. Zealand J. Sc. *35*(A): 67–92.

——— 1953b. Studies in monozygotic cattle twins. VIII. Performance of identical twins when uniformly treated after having been subjected to different treatments in the previous lactation. N. Zealand J. Sc. *35*(A): 93–96.

JOHANSSON, I. 1953. The manifestation and heritability of quantitative characters in dairy cattle under different environmental conditions. Acta genet, Basel. *4*: 221–231.

——— 1954. An analysis of data from the Danish bull progeny testing stations Zschr. Tierzücht. Züchtungsbiol. *63*: 105–126.

——— 1955. The first lactation yield as a basis of selection as compared with the second and third lactations. Proc. Brit. Soc. Animal Prod., pp. 102–108.

JOHANSSON, I., and O. CLEASSON. 1957. Factors affecting the composition of milk. *In* Progress in the Physiology of Farm Animals, ed. J. HAMMOND, vol. 3, pp. 1005–1043. London: Butterworth.

JOHANSSON, I., and A. HANSSON. 1940. Causes of variation in milk and butterfat yield of dairy cows. Kungl. Lantbr. Akad Tidskr. *79*(6½): 1–127.

LEGATES, J. E. 1957. Heritability of fat yields in herds with different production levels. J. Dairy Sc. *40*: 631.

LEGATES, J. E., and J. L. LUSH. 1954. A selection index for fat production in dairy cattle utilizing the fat yields of the cow and her close relatives. J. Dairy Sc. *37*: 744–753.

LUSH, J. L., and E. N. SHULTZ. 1936. Heritability of butter fat percentage and butterfat production in the data with which sires have been proved in Iowa. J. Dairy Sc. *19*: 429–430.

MAHADEVAN, P. 1951. The effect of environment and heredity on lactation. I. Milk yield. J. Agr. Sc. *41*: 80–88.

MASON, I. L., and A. ROBERTSON. 1956. The progeny testing of dairy bulls at different levels of production. J. Agr. Sc. *47*: 367–375.

RENDEL, J. M.; A. ROBERTSON; A. A. ASKER; S. S. KISHIN; and M. T. RAGAB. 1957. The inheritance of milk production characteristics. J. Agr. Sc. *48*: 426–432.

ROBERTSON, A., and A. T. G. MCARTHUR. 1955. Genetic differences between bull-breeding herds. Proc. Brit. Soc. Animal Prod., pp. 94–101.

ROBERTSON, A., and I. L. MASON. 1956. The progeny testing of dairy bulls: A comparison of special station and field results. J. Agr. Sc. *47*: 376–381.

SANDERS, H. G. 1927–8. The variations in milk yields caused by season of the year, service, age, and dry period, and their elimination. Part I. Season of the year. J. Agr. Sc. *17*: 339–379. Part II. Service. *17*: 502–523. Part III. Age. *18*: 46–67. Part IV. Dry period and standardization of yields. *18*: 209–251.

TYLER, W. J., and G. HYATT. 1947. The heritability of milk and butterfat production and percentage of butterfat in Ayrshire cattle. J. Animal Sc. *6*: 479–480.

SYMPOSIUM

MUTATION AND MUTAGENESIS

1. H. STUBBE. Advances and Problems of Research in Mutations in the Applied Field
2. NORMAN H. GILES. Mutations at Specific Loci in *Neurospora*
3. EVELYN M. WITKIN. Post-irradiation Metabolism and the Timing of Ultraviolet-induced Mutations
4. C. P. SWANSON, T. MERZ, and N. COHN. Metabolism and the Stability of Chromosomes
5. H. J. MULLER. The Mutation Theory Re-examined

ADVANCES AND PROBLEMS OF RESEARCH IN MUTATIONS IN THE APPLIED FIELD

H. Stubbe[1]

SOON AFTER Muller, more than thirty years ago, provided evidence that ionizing radiations increase the frequency of mutations, the question arose as to the extent to which this discovery could be applied in breeding work. In the United States there were at first no such studies, largely because of Stadler's criticism of this work. Only during recent years has the attitude changed, and today mutation experiments are being carried out with many cultivated plants in the United States.

In Europe, especially in Sweden, the Soviet Union, and Germany, the producing of mutants of high value for breeding purposes by experimental means began early. In 1929 Nilsson-Ehle started such work and later on it was continued by Gustafsson. Twenty-five years ago valuable starts along this line were also made in the Soviet Union, especially by Delaunay and Sapehin, although these studies have unfortunately been interrupted. In Germany since 1929 we have repeatedly studied those aspects of experimental mutation which might be important for the production of varieties of high breeding value in cultivated plants. Experiments on barley, started in 1932 in co-operation with Kuckuck at the Kaiser Wilhelm Institut für Züchtungsforschung at Müncheberg, had to be interrupted in 1936 and it was not until the end of World War II that they could be resumed, by myself and my colleagues at the Institut für Kulturpflanzenforschung (Institute for Cultivated Plants Research) at Gatersleben. Meanwhile, Freisleben and Lein at Halle had started studies on barley, which are being carried on successfully by Walter Hoffmann.

It is not my task to report in detail on the numerous achievements of mutation research in cultivated plants. The new method of producing mutants has made it possible to treat not only higher plants, but also microbes. For instance, in the large-scale industrial production of penicillin in the United States and England efforts have been made for a long time to reduce costs of production by isolating *Penicillium* strains which give greater yields. Since the submerged-culture method has come into vogue, extraordinarily valuable mutations have been induced in *Penicillium,* using the experience gained from working with biochemical mutants of *Neurospora.* I remind you of the fundamental experiments of Demerec, Backus, and others. In *Actinomyces* Kelner succeeded in isolating mutants of high antibiotic activity out of an antibiotically inactive strain by X-ray irradiation. Recently, Prokofieva-Belgovskaya *et al.,* of the Institute of Biophysics and the Institute for Antibiotics Research in Moscow, have reported on X-ray induced mutants, likewise in *Actinomyces,* which are clearly superior to the original strain in the production of streptomycin. In the United States, by inducing mutants in *Aspergillus terreus,* a further increase in the production of itaconic acid has been achieved.

The production of superior mutants in the industrially important micro-organisms has thus been a highly significant economic success. However, so far there have been only a few publications on this work, which is mostly performed in

[1]Institute for the Study of Cultivated Plants, German Academy of Sciences, Gatersleben, Germany.

industrial laboratories. We may expect that many further results will be reported which will confirm the usefulness of this method.

In the evolution of cultivated plants, under the control of man, new gene recombinations have been produced making the species or populations maximally adapted to the requirements of man. In the process of controlled evolution we must no longer neglect the possibilities offered by experimental mutation research. It will in the future be of equal importance with the collection and preservation of the world resources of cultivated plants and of their near relatives, since it will offer the possibility of creating entirely new cultivated plants.

To estimate the breeding value of the mutants of our crop plants, field trials are of the greatest importance. Results of such yield tests made on a large scale, carried out for several years, and, as much as possible, under different conditions of growth, have so far been published mainly in Sweden. Gustafsson in 1947 and Fröier in 1954 have reported on such experiments on barley, and MacKey in 1954 on mutants of wheat and oats. In Germany, in 1951, Hoffman reported on barley mutants tested at Halle over a period of several years.

The results of these field trials are highly encouraging and give evidence of the diverse opportunities offered by mutation breeding. The tested mutants generally exhibit characteristics which constitute an improvement with regard to special breeding objectives. In yield many of these mutants are equivalent or even superior to the original varieties. In Sweden two mutant strains of cross-fertilizers, the white mustard (*Sinapis alba*) and the summer oil rape, have been developed into market varieties. Furthermore, in Sweden a pea mutant, and in Germany and the United States a bean mutant (*Phaseolus vulgaris*), are being marketed. In barley the erectoides mutants, which are of considerable value for breeding work, have been intensely studied in Sweden. They are marked by high stiffness of the straw, resulting in better adaptation to nutrition at a high nitrogen level and thus in better yields. For this reason they were termed "nitrogen ecotypes" by Gustafsson. Mutants carrying new alleles for resistance to different diseases are of particular value. Barley mutants resistant to mildew have repeatedly been found. In the United States workers have produced selected mutants, both induced and spontaneous, of oats and wheat which are resistant to several fungi. Moreover, alleles have been found for resistance to rust in flax and to leaf spot and stem rot in peanuts. Within the last few years there have also been successful experiments with vegetatively propagated plants as, for instance, fruit trees, potatoes, and ornamental plants.

That chromosomal aberrations may also lead to strains of high breeding value has been shown by Nybom (1954) in barley and by Sears (1956) in wheat. Bushland and co-workers (1956) utilized the lethal effect of most of the chromosomal aberrations in the struggle against the screw-worm fly, a pest of cattle and goats in the tropics and subtropics.

I regret that I could cite here only a few examples to illustrate the importance of applied mutation research. Now I wish to present some particular results, which have been achieved since 1946 with different plant species in the Institute for Cultivated Plants Research of the German Academy of Sciences at Gatersleben.

Everyone engaged in mutation research on cultivated plants knows from experience that only a very small proportion of mutants are desired types. This fact induced some workers to reject the use of radiation in plant breeding. However, this attitude neglects some essential aspects of this method.

First, mutation experiments are generally carried out with high-yielding varieties of cultivated plants; therefore, it is not to be expected that improved forms will

arise in great quantity. In such idiotypes the production of desirable alleles involves an abbreviation of the breeding procedure because already in the X_2 and the F_2 generations homozygous segregates will be found. In recombination breeding it takes much more time to obtain homozygous lines and high-yielding populations. Different mutated alleles can be combined by crossing without difficulties, and they can be obtained as homozygotes already present in the F_2. In mutation research on cultivated plants very great significance is to be ascribed to such combinations.

In ancient high-bred cultivated plants a productive variety with additional promising characteristics can thus be produced without the necessity of protracted backcrossing. In recently cultivated plants characteristics can be induced which in spontaneously varying material are either absent or very rare. Valuable characteristics can likewise be added to the highly heterozygous clones of vegetatively propagated plants which hardly permit purposeful recombination breeding. Last but not least, close linkage can be broken and little segments of a chromosome can be transferred to a non-homologous chromosome.

Secondly, not all the mutants that effect pathological disturbances in the original variety are, from the first, useless for breeding work. Combined with a favourable idiotype they may lose their pathological effects and become valuable for breeding purposes. For example, in the snapdragon, *Antirrhinum majus,* which has been the object of numerous theoretical experiments, a mutant, *eramosa,* has been induced which has nearly lost the ability to branch. Moreover, considerable pathological disturbances exist in the flowering region, for instance, inhibition of flower formation and deformation of the flowers. A characteristic of breeding value is, however, manifested by this pathological mutant: the lack of branching produces an erect growth, a characteristic desired for a long time in breeding ornamental plants. One of our best-known German breeders of ornamental plants, Mr. Vogel of the Institute for Plant Breeding at Quedlinburg, has succeeded in producing a snapdragon of tall growth with a single stem. This new snapdragon form has been achieved by outcrossing the pathological mutant to another variety. In the outcross the pathological alterations of the inflorescences are normalized by the changed idiotypic background. The pleiotropic complex, which had been present, is now broken. The lack of branching is preserved; the flowering region is normal.

Thirdly, the great number of pathological mutants useless for breeding work is nevertheless of high importance for theoretical studies. For studies in physiology, developmental physiology, and biochemistry of plants our mutant collections represent inexhaustible sources and may aid in understanding gene action. The mutants are likewise of great importance to research on morphology, ontogenesis, and systematics, as well as to the increase of our information on the genetics of the plants. For instance, within the collection of barley mutants, we can observe the reappearance of the whole range of natural variants in the genus *Hordeum.* This fact is of outstanding value for our understanding of the variability in this genus.

Fourthly, forms valuable for breeding purposes have been selected out of the progenies of organisms which have been treated with ionizing radiations. This fact —and I wish to state this very clearly—must, of course, not be misinterpreted, as a famous atomic physicist is said to have recently done. He said that there would not be very much danger for the human population from radiation because valuable mutations would be induced. This wrong inference is very important, the more so as it belittles dangers that can have serious consequences for mankind if we do not work against it in time. In our experimental subjects we are always able to reject the pathological forms or to experiment with them as we wish. In man, however,

we are bound by the laws of ethics which forbid any experiments. Therefore, we geneticists have the duty to warn people most emphatically against all mutagenic agents to which man is exposed.

After this introduction let me return to the mutation experiments on cultivated plants at Gatersleben.

At first we set up experiments on spring and winter barley. Dormant seeds were treated with X-rays. The present collection of X-ray induced mutants includes about eight hundred lines. A great many of them have been used in investigations of their genetics, morphology, physiology, and so on. Central in our work, however, were those forms which are of special interest to the breeder. Thus, within the last few years, yield trials have been performed with smooth-awned, naked-kernelled, early, large-kernelled, and stiff-strawed mutants. In all about two hundred different mutant lines have been tested in yield trials. Some of them have been tested for one or two years only since some of these mutants were recently selected, or because in their first year defects were already apparent which made further testing unpromising. My collaborator, F. Scholz, who conducts these investigations, has recently reported on the yield trials of more than a hundred mutants of spring and winter barley. The most important results up to 1957 are summarized in Tables I

TABLE I

GRAIN YIELDS OF X-RAY INDUCED MUTANTS IN TWO-ROWED SPRING BARLEY*

Mutant	Serial no. of mutants	Number of years tested	Relative value of grain yield (mother line = 100) on an average
Erectoides	2,654	6	105.7
Erectoides	2,660	5	105.1
Erectoides	3,229	2	104.3
Erectoides	2,671	3	103.4
Erectoides	4,013	3	101.0
Erectoides	3,211	3	100.6
Erectoides	2,661	4	100.2
Erectoides	4,009	3	99.5
Erectoides	4,018	2	98.5
Erectoides	3,061	3	97.7
Erectoides	3,059	5	97.3
Naked-kernelled	4,129	6	101.8†
Naked-kernelled	3,041/6a	4	95.7†
Semi-smooth-awned	3,945	6	112.6
Semi-smooth-awned	3,943	4	110.7
Semi-smooth-awned	3,946	4	107.7
Semi-smooth-awned	3,042	3	102.8
Smooth-awned	4,033	5	108.0
Early (3 days)	3,706	5	97.7
Large-kernelled	3,705	4	99.6
Large-kernelled, early (6 days)	4,242	3	103.5
Large-kernelled, early (5 days)	3,887	3	97.8
Large-kernelled, early (3 days)	3,746	5	96.6
Large-kernelled, early (4 days)	3,741	4	96.2
"High-yielding"	3,635	8	103.3
"High-yielding"	2,590	5	102.8
"High-yielding"	2,636	4	102.5

*Field trials at Gatersleben, 1950–7.
†Hulls inclusive.

TABLE II

GRAIN YIELDS OF X-RAY INDUCED MUTANTS IN SIX-ROWED WINTER BARLEY*

Mutant	Serial no. of mutants	Number of years tested	Relative value of grain yield (mother line = 100) on an average
Early (4 days)	506	4	115.0
Early (4 days)	481	5	105.6
Early (4 days)	505	4	104.4
Early (8 days)	941	4	103.0
Early (5 days)	969	3	102.3
Early (4 days)	496	4	101.4
Mildew-resistant	515	4	119.1
Mildew-resistant	520	4	113.1
Mildew-resistant	511	4	112.8
Mildew-resistant	512	3	108.0
Tall-strawed, late	818	3	109.4
Tall-strawed, late	829	2	103.9
Tall-strawed, late	803	2	100.0
Broad-leafed	507	4	107.4

*Field trials at Gatersleben, 1950–7.

and II. In spring barley, mutants of the varieties Haisa and Donaria, both two-rowed, and in winter barley, mutants of the six-rowed varieties Friedrichswerther Berg, Peragis Mittelfrühe II, and Kleinwanzlebener 12, are involved. The stiff-strawed erectoides mutants of the two-rowed spring barley were very satisfactory (see Table I), in full agreement with the observations of other authors. The superior straw-stiffness of these lines is unquestionable. It seems to me that use of these forms would open the possibility of obtaining new high-yielding varieties of superior straw-stiffness. These varieties surpass the original varieties in yield, especially when highly supplied with nitrogen.

The naked-kernel mutants of spring barley likewise are of considerable importance. They offer a very instructive example of possible induced changes of single characteristics while preserving all other characteristics of the mother line. The superiority of the naked barley is based mainly on its improved nutritional value in fattening pigs. In order to breed productive naked barleys the breeders in Central Europe were previously dependent on transferring the allele *n* for nakedness from primitive or non-adapted forms to high-yielding European varieties. But in spite of intense work for many years all such attempts proved unsuccessful. Our naked mutants, however, have the same yield as the mother lines and do not differ from them, except in their nakedness (Scholz, 1955).

In the Gatersleben semi-smooth or smooth-awned mutants similar results have been obtained. The yields of such lines are, on the average, even greater than those of the original varieties. Scholz (1958) is working on some special problems concerning these mutants and in the course of the Congress an account will be given of these studies.

Some other mutants, which do not show distinct changes compared to the original varieties and which have been selected only because of their promising appearance, have given favourable results too. We call them "high-yielding."

Large-kernelled mutants, however, have been less successful and are not promising. Likewise the early ripening mutants of spring barley very rarely show satisfactory yields.

In contrast to the early mutants of spring barley the mutants of winter barley (Table II), which are as much as eight days earlier in heading and up to five days earlier in ripening, have had at least the same, and very often even better, yields than the mother lines. These diverse reactions in spring and winter barley are probably due to differences in their growth habits and to local climatic conditions. The experiments, however, demonstrate that the use of well-adapted mutants is promising if under certain circumstances breeding of early varieties is desirable.

Excellent results have been achieved with mildew-resistant mutants of winter barley. Because of their high resistance they produce high yields, especially in wet years when infection with mildew is heavy. In this respect 1956 has been typical: compared with the original varieties the mutants showed grain yields of 130 per cent on the average. All mutants cited in Table II have the same dominant allele "Resistens"; another mutant, not mentioned here, carries the allele "Obsistents" (Nover and Bandlow, 1958). Using them it was possible to differentiate new mildew races (Nover, 1957).

During the last few years experiments have been carried out to analyse series of X_2 plants and their progenies in order to discover types containing a higher content of crude protein than the mother lines. In these analyses (after Kjeldahl), the Müncheberger Method (after Schwarze) has been employed.

So far there have been analysed on the average about 8,000 X_2 plants of winter barley and about 17,000 X_2 plants of spring barley per year for their crude protein content. The work is difficult because of the very high variability of protein content under the influence of environmental factors. Therefore, we have to carry out these analyses with a large amount of material and for many years.

In twelve lines of spring barley originating from X_2 plants for three consecutive years (that is to say in the X_4, X_5, and X_6 generations), the protein content has been superior to that of the mother lines. In some cases these increases are only small, in several cases, however, they are remarkable. The results from the four best lines have been summarized in Table III. There is hardly any morphological difference between these lines and the original varieties. Two among them are X-ray

TABLE III

CONTENTS OF CRUDE PROTEIN OF HIGH PROTEIN MUTANTS IN BARLEY COMPARED WITH THE CONTROLS (MOTHER LINES)

Mother line (control)	Mutant	1955 (%)	1956 (%)	1957 (%)	Average (%)
Spring barley variety Freya	St. 5830	13.7	15.6	15.8	15.0
	Control	11.2	12.5	12.2	12.0
	Difference	+ 2.5	+ 3.1	+ 3.6	+ 3.0
	St. 5831	14.8	16.2	15.2	15.4
	Control	12.2	12.9	11.1	12.1
	Difference	+ 2.6	+ 3.3	+ 4.1	+ 3.3
Mutant 4129 nudoides (X-ray induced mutant from the spring barley variety Haisa)	St. 5707	16.9	16.6	15.7	16.4
	Control	12.7	14.5	13.4	13.5
	Difference	+ 4.2	+ 2.1	+ 2.3	+ 2.9
	St. 5708	15.1	16.2	15.3	15.5
	Control	12.6	14.5	13.4	13.5
	Difference	+ 2.5	+ 1.7	+ 1.9	+ 2.0

induced mutants of the variety *Freya* (var. *nutans*). The remaining two lines have naked kernels; they arose following repeated X-irradiation of an X-ray induced naked mutant of the covered spring variety *Haisa*. The content of crude protein in some protein mutants is up to 25 per cent higher than in the original varieties. The reported values for the crude protein content for each year constitute the mean values of three to five replicated plots, each compared to neighboring control plots. In more recent material there are several additional types that may have a superior protein content, but statements cannot yet be made with certainty. In contrast to spring barley, analyses of winter barley so far have had no success.

For the present no conclusive information has been obtained about the yielding capacity of the protein mutant lines, since often results for only one year are available. The mutants with high protein content, however, seem generally to have smaller yields than the original varieties. In the few experiments made so far the relative yields of these mutants ranged from 80 to 90 per cent approximately. Experiments, which will extend over a period of several years and use more material, will give reliable information. We cannot yet decide whether or not the high protein content of these lines is controlled by a single mutated gene.

Another object of our research is the soy bean which is being studied by my colleague M. Zacharias. Under the climatic conditions of Germany the early ripening of this valuable crop is of particular importance for the increase and the reliability of the yield. Only when this breeding objective is attained, will the soy bean become an important cultivated crop in Germany. At present we have about three hundred different mutant types at Gatersleben. Mutants with high numbers of pods and with a shortened vegetative period selected in 1953 and 1954 were abundantly propagated in the following years in order to obtain enough seeds for larger yield tests. The tests carried out in 1957 confirm the supposition that the mutant strains, in part morphologically clearly different from the original variety, are superior in yield and some of them five to seven days earlier (see Table IV). Another means of obtaining reliable yields is a lengthening of the vegetative period, the harvest time remaining the same as before. For this purpose lines are selected which germinate at a lower temperature than the mother line and which therefore can be sown at an earlier date. The normal temperature of germination being 6 or 6.5° C., we succeeded in bringing to germination some few seeds among thousands by selection in the climate room at 4.5° C. Out of these seeds lines have been produced which, germinating about six days earlier, have an advantage in growth that persists till

TABLE IV

RESULTS OF YIELD TEST OF SOME MUTATION STRAINS OF SOY BEAN IN RELATION TO THE ORIGINAL VARIETY

Test no.	Kg./trial plot	Quintal/ hectare	Relative	d	t	*P* (%)	Vegetation period (days)
Heimkraft I	1.807	9.04	100	—	—	—	154
Mutation strain							
C–V 79	2.107	10.54	116.59	+0.300	25.00	<0.10	157
C–V 107	1.928	9.64	106.64	+0.121	10.08	<0.10	149
C–V 168	1.850	9.25	102.32	+0.043	3.58	<0.10	149
C–V 177	1.897	9.49	104.98	+0.090	7.5	<0.10	156
C–V 181	2.030	10.15	112.28	+0.223	18.58	<0.10	147
C–V 190	2.190	10.95	121.19	+0.383	31.92	<0.10	155

maturity (Fig. 1). Early germination, however, does not always imply early maturity (see strain 9), it may also involve a delay in ripening. Investigations of this behavior have been started.

Our research on the soy bean has thus proved that by means of experimentally induced mutants the two most important breeding objectives have been attained, namely, increase of yield and reliability of yield. The absolute values, however,

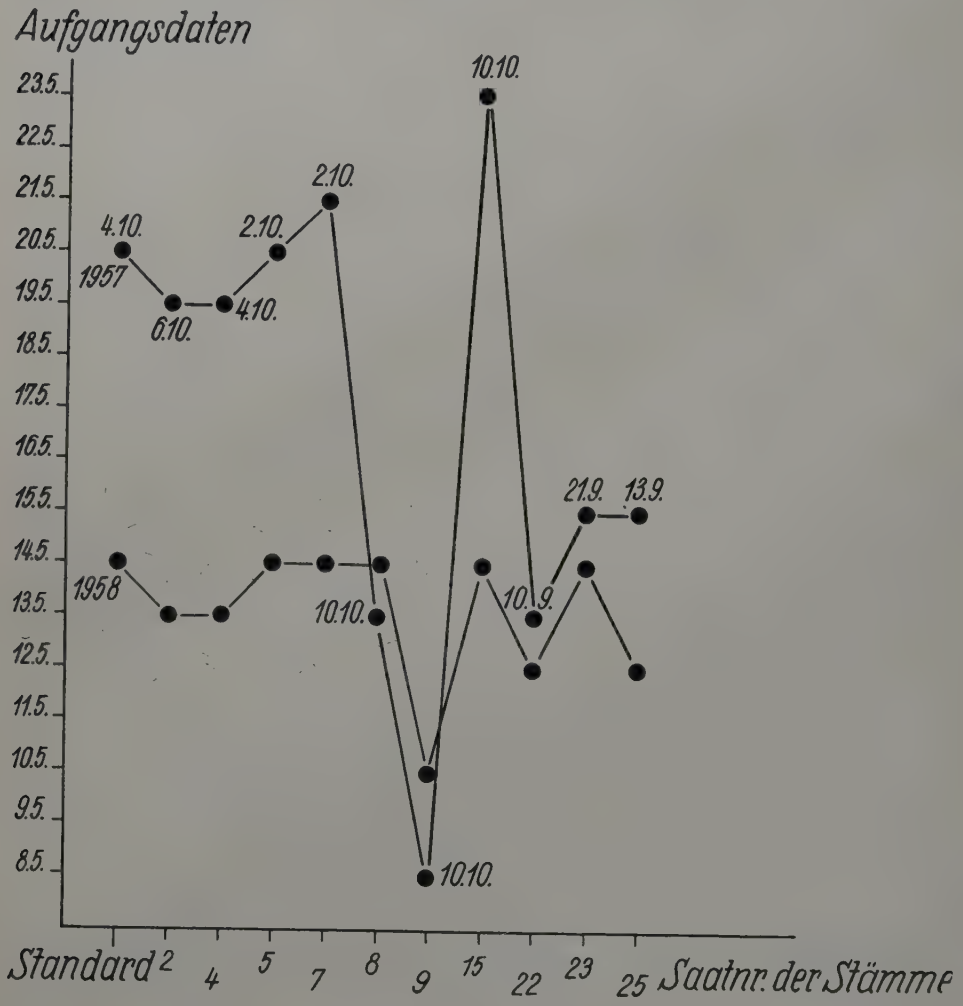


FIGURE 1

are not always a sufficient basis for extending the cultivation of this valuable crop plant to the climatic conditions of Germany. Some of the mutants, however, are of great value as basic material for recombination breeding, for instance, with early and high-yielding Chinese varieties collected in 1956 in northeast China.

Very extensive mutation experiments have furthermore been carried out with the cultivated tomato, *Lycopersicon esculentum* and concomitant with the wild form *Lycopersicon pimpinellifolium*. At present about three hundred mutants of *Lyco-*

persicon esculentum and two hundred and fifty mutants of *Lycopersicon pimpinellifolium* have been obtained at Gatersleben. Experiments are being carried out on wild forms of the genus *Lycopersicon* and likewise on the genus *Hordeum* to elucidate the problem of production of parallel mutations and other related problems. However, I do not want to report on these studies here.

There are many possibilities for theoretical and applied research on the mutants of the cultivated tomato. We are working on the important problem of early maturity, simultaneously preserving high yield and a uniform and good quality of the fruits. Many early mutants have been produced, and have been crossed with all early varieties available to us, and this year we have grown a great number of F_3 lines originating from early F_2 plants. I am sorry that at present no exact information can be given about the results of this experiment.

However, I wish to mention the first results of an experiment, in co-operation with Dr. Schreiber, alkaloid chemist in the Institute of the German Academy of Agricultural Sciences, for research in control of the Colorado beetle at Mühlhausen. These investigations are of interest to alkaloid chemistry and of importance for practical purposes.

Since the fundamental studies of Fontaine and his colleagues, we know that all species of *Lycopersicon* analysed to date contain the alkaloid tomatine in varying quantities. Chemically, tomatine belongs to the group of the steroid alkaloid glycosides. It is composed of an aglycone portion, tomatidine, and a tetrasaccharide moiety consisting of two molecules of glucose and one each of galactose and xylose. β-tomatine is free of xylose and, therefore, contains only a trisaccharide portion. Since its discovery it has increasingly gained an important position and because of its special qualities it has aroused interest in quite different fields of research. The resistance to the Colorado beetle of most of the *Lycopersicon* species and of some wild potatoes for instance is due to the presence of certain quantities of tomatine within the leaves of these plants. *In vitro* tomatine inhibits the development of numerous micro-organisms. All data indicate that phytopathogenous micro-organisms are only insignificantly injured by this alkaloid, certain dermatophytes, however, to a high degree. Nevertheless, interest in *Solanum* and *Lycopersicon* alkaloids on the part of the pharmaceutical industry has quite a different explanation. With high yield tomatidine as well as some other *Solanum* alkaloids can be chemically degraded into pregnane derivatives which are employed as intermediate products in the industrial synthesis of the therapeutically important sex and adrenal cortical hormones. Moreover, like digitonine, which is expensive and cannot be easily derived, tomatine is particularly suited to the selective precipitation of different steroids. In future, therefore, a higher demand for this alkaloid is to be expected, especially for routine sterol determinations in clinical laboratories.

Thus, it is clear that for the last few years there have been thorough studies on the pharmacology and toxicology of tomatine and its derivatives as well as on the chemistry and biochemistry of this alkaloid. Even so we have no accurate information yet about the biogenesis of tomatine nor connected problems of plant physiology.

The Gatersleben collection with its numerous experimentally induced mutants of *Lycopersicon esculentum* and *Lycopersicon pimpinellifolium* provided the opportunity for studying some problems of the biosynthesis of tomatine. In 1957 experiments were made on the alkaloid chemistry of about a hundred mutants.

To sum up, we can state that the alkaloid content of most of the analysed mutants differs not at all or only slightly from the controls, both quantitatively and qualita-

tively. Some mutants, however, were relatively poor in alkaloids compared with the controls; others, especially many mutants of *Lycopersicon pimpinellifolium* (see Tables V-VIII) show a strikingly high percentage of alkaloids in their leaves. Interrelations between the rate of alkaloid synthesis on the one hand and chlorophyll formation within the leaves on the other have not been observed. Generally,

TABLE V

ALKALOID CONTENT OF THE LEAVES OF SOME MUTANTS IN *Lycopersicon esculentum* (VARIETY: "CONDINE RED")

No.	Designation	Percentage of alkaloid in dry matter	Relative value of alkaloid (control = 100)	To.	β-To.	De.	β-De.*
22/57	Condine Red-Kontrolle	0.64	100	+	+		
3/57	*hemiglobosa*	0.23	36	+	(+)		
39/57	Heller, feinfiedriger Zwergbusch	0.40	63		+		(+)
88/57	Mittelgr.Pflze.m.gekielten Fied.	1.05	164	+	(+)		
95/57	Schwache, dunkle Pflanze	1.53	239	+			
104/57	Zierliche Pflanze	0.26	41	+	(+)		
119/57	Aufgehellte Pflanze	1.10	172	+			

*To. = Tomatine; β-To. = β-Tomatine; De = Demissine (?); β-De. = β-Demissine (?).

TABLE VI

ALKALOID CONTENT OF THE LEAVES OF SOME MUTANTS IN *Lycopersicon esculentum* (VARIETY: "LUKULLUS")

No.	Designation	Percentage of alkaloid in dry matter	Relative value of alkaloid (control = 100)	To.	β-To.	De.	β-De*
36/57	Lukullus-Kontrolle	0.82	100	+	(+)		
175/57	Schmale Fiedern	1.28	156	+		(+)	
206/57	Dürreempfindlich	1.27	155	+	(+)	(+)	
231/57	Kleistogame Blüten	0.44	54	+	(+)		
246/57	*prunoidea*	0.81	99		+		

*To. = Tomatine; β-To. = β-Tomatine; De. = Demissine (?); β-De. = β-Demissine (?).

TABLE VII

ALKALOID CONTENT OF THE LEAVES OF SOME MUTANTS IN *Lycopersicon esculentum* (VARIETY: "RHEINLANDS RUHM")

No.	Designation	Percentage of alkaloid in dry matter	Relative value of alkaloid (control = 100)	To.	β-To.	De.	β-De.*
276/57	Rheinlands Ruhm-Kontrolle	1.11	100	+	+		
273/57	Gelbl.-grüne Pflze.m.schlecht. Ansatz	0.52	47	+	(+)	(+)	
287/57	Sprosse gekrümmt, fast kriechend	1.45	130	+			
307/57	Blätter kurz, glänzend, stark gekrümmt	0.79	71	+		+	
308/57	Ältere Blätter gerollt	0.44	40	+	(+)	+	(+)
328/57	*lurida*	0.61	55	+	(+)		

*To. = Tomatine, β-To. = Tomatine, De. = Demissine (?), β-De. = β-Demissine (?).

TABLE VIII

ALKALOID CONTENT OF THE LEAVES OF SOME MUTANTS IN *Lycopersicon pimpinellifolium*

No.	Designation	Percentage of alkaloid in dry matter	Relative value of alkaloid (control = 100)	To.	β-To.	De.	β-De.*
P 1/57	Pimp.-Kontrolle	1.00	100	+	(+)		
P 19/57	Dicht gefiederte Pflanze	1.96	196	+	+	(+)	(+)
P 44/57	Kriech.Pflz.m.langgestielt. Bl.u.Fied.	2.14	214	+			
P 76/57	Kleiner dichter Busch	0.55	55	+	(+)	(+)	
P 79/57	Kleine hellgrüne Pflanze	0.50	50	+	(+)		
P 81/57	Lockerer kleiner Busch m.oval.Fied.	0.44	44	+			
P 125/57	Heller Busch	0.76	76	(+)		+	(+)
P 132/57	Hellgrün	2.82	282	(+)		+	
P 177/57	Graugrüne, dichtsitzende Fiedern	1.52	152	(+)		+	
P 188/57	Stark Anthozyan-halt.,anfangs dicht belaubt.	0.73	73	+		+	
P 201/57	Junge Fiedern gekraust	1.53	153	+	(+)	+	(+)
P 205/57	Mikado-Blatt	2.43	243	+		(+)	
P 208/57	Dunkelgrüne, dicht gefied. Pflanze	1.33	133	+	+	+	+
P 213/57	Kräftige Pflanze m.blasigen Fiedern	2.38	238	+	(+)	(+)	
P 216/57	Fiedern fast ganzrandig	2.27	227	+	(+)		

*To. = Tomatine; β-To. = β-Tomatine; De. = Demissine (?); β-De. = β-Demissine (?).

there has been more alkaloid in slowly growing mutants than in those of strong growth. This is a fact that has been repeatedly confirmed in analyses with other alkaloids and plants.

Chemical analyses of individual alkaloids have shown surprising results. In several mutants, in addition to the well-known tomatidine glycosides (tomatine + β-tomatine), there have been demonstrated sometimes in considerable quantities, glycosides of an alkaloid with a tertiary nitrogen atom. Tomatidine, as you know, has a secondary amino group. The newly isolated main alkaloid which had not been previously found in tomatoes has been degraded to the same four carbohydrates like tomatine, but to a different aglycone, namely to the tertiary base demissidine. Therefore, the new tetraoside is supposed to be identical with demissine. Similarly some mutants with small amounts of β-demissine, corresponding to β-tomatine, may be found.

At present it cannot be definitely stated that the formation of demissine is primarily due to a mutational disturbance of the alkaloid metabolism. It would be conceivable that, in general, demissidine glycosides are formed as main alkaloids in the vegetative phase, tomatidine glycosides during the reproductive phase. If this is true the demissidine appearing in some mutants at the end of the vegetative period in contrast to the controls, would be an indicator of the probably specifically disturbed general development of the plants. The differences in the relative quantities of the alkaloids of the mutants might be explained similarly. Systematic analyses during the vegetative period will supply this information.

From a biochemical point of view a direct conversion of demissidine into tomatidine is most unlikely. It is more probable that the biosynthesis of both steroid alkaloids starts from the same precursor and then proceeds along parallel lines; depending on the specific vegetation stages and developmental conditions one pathway of synthesis or the other is preferred.

In two mutants (see Table V, no 39/57 and Table VI, no 246/57) probably genic disturbances effecting the biosynthesis of the carbohydrate components

of the alkaloid glycosides were observed. In the two plants only glycoalkaloids were isolated, which in contrast to tomatine and demissine proved to be free of xylose (β-tomatine, in no. 39/57 there were also small amounts of β-demissine).

We hope that the above-mentioned analyses of tomato mutants will contribute to the elucidation of the biogenesis of the steroid alkaloid glycosides in these plants. Moreover, mutants with a high tomatine content and particularly high tomatine yield might be found. The *Lycopersicon pimpinellifolium* mutants, P 44/57, P 205/57, P 213/57, and P 216/57, seem to meet these conditions because there is an increase in the tomatine content of more than 100 per cent compared with the controls. These plants would be especially suited for the extraction of the important alkaloid.

Finally, our mutation experiments with fruit trees, started in 1954, have already produced their first results. For the grafting of four valuable apple varieties, namely Transparent Blanche, James Grieve, Ontario, and Gravenstein, 3,006 scions and 2,075 buds have so far been irradiated and grafted on trees eight to nine years old. These experiments are being carried out by my colleague, Gröber. To date we have observed a great number of variations in maturation, fruit shape, size, and colour, and in the cavities of peduncles and calyx. At the present stage of these studies no definite statement is possible as to whether these changes are really based on mutations. However, it can be seen that in the variety Transparent Blanche, for instance, the red-coloured fruit appeared at a rate of 5.5 per cent if the number of the irradiated buds and the occurrence of mutants is taken as a basis of calculation. For more than twenty years breeders have been searching for this variant.

By means of experimental mutation research it is possible to produce in all cultivated plants numerous and very different types which make possible the achievement of certain desired breeding objectives or at least decisive progress in this direction. By certain induced changes a qualitative improvement of the cultivated plants is produced. Preservation of the productivity of the original varieties can be widely realized. In some plants even simultaneous improvements of yield are possible. The special value of the induced mutants is due to their favourable idiotypical constitution if they have been derived from appropriate original varieties. By establishing extensive collections of mutants, based on productive varieties, excellent sources of valuable material for theoretical research and for breeding work can be provided. Without being compelled to employ the troublesome and protracted backcrossing procedure, a diversity of new recombinations of characteristics can be achieved by single crosses which may involve the simultaneous realization of different breeding objectives. After production and testing of valuable mutants such recombination crosses between different mutants should always follow. From our experience we are of the opinion that the value of mutation-breeding as a modern method of plant-breeding has been justified. Increases in productivity, which are attainable by this method, can hardly be obtained by the standard methods of plant-breeding, especially in highly bred cultivated plants.

REFERENCES

Backus, M. P., J. F. Stauffer, and M. J. Johnson. 1946. Penicillin yields from new mold strains. J. Am. Chem. Soc. *68*: 152–153. Cited from Raper and Thom (1949).

Bandlow, G. 1951. Mutationsversuche an Kulturpflanzen. II. Züchterisch wertvolle Mutanten bei Sommer- und Wintergersten. Züchter *21*: 357–363.

——— 1954. Mutationsversuche an Kulturpflanzen. III. Ueber genetische Vorstufen der Kapuzengerste mit variabler Manifestierung bei röntgeninduzierten Mutanten. Züchter *24*: 20–27.

BUHR, H., R. TOBALL, und K. SCHREIBER. 1958. Die Wirkung von einigen pflanzlichen Sonderstoffen, insbesondere von Alkaloiden, auf die Entwicklung der Larven des Kartoffelkäfers (*Leptinotarsa decemlineata* Say). Ent. exp. & appl. *1*: 209–224.

BUSHLAND, R. C., E. F. KNIPLING, and A. W. LINDQUIST. 1956. Eradication of the screwworm fly by releasing gamma-ray-sterilized males among the natural population. Proc. Internat. Conf. Peaceful Uses Atomic Energy *12*: 216–220.

DELAUNAY, L. N. 1931. Resultate eines dreijährigen Röntgenversuchs mit Weizen. Züchter *3*: 129–137.

——— 1934. Experimentell erzeugte Mutationen bei Weizen. Verlag Ukrain. Akad. Landw. Wiss. S. 1–56 (Russian).

FONTAINE, T. D., G. W. IRVING, Jr., R. MA, J. B. POOLE, and S. P. DOOLITTLE. 1948. Isolation and partial characterization of crystalline tomatine, an antibiotic agent from the tomato plant. Arch. Biochem., N.Y. *18*: 467–475.

FONTAINE, T. D., P. S. SCHAFFER, H. M. DOUKAS, W. E. SCOTT, R. M. MA, V. A. TURKOT, F. DEEDS, R. H. WILSON, and S. P. DOOLITTLE. 1955. Tomato leaves a potential alkaloid and sterol source. U.S. Agr. Res. Serv., ARS-73-8, pp. 23.

FREISLEBEN, R., and A. LEIN. 1942. Ueber die Auffindung einer mehltauresistenten Mutante nach Röntgenbestrahlung einer anfälligen reinen Linie von Sommergerste. Naturwissenschaften *30*: 608.

——— 1943. Möglichkeiten und praktische Durchführung der Mutationszüchtung. Kühn-Arch. *60*: 211–225.

——— 1944. Röntgeninduzierte Mutationen bei Gerste. Züchter *16*: 49–64.

FRÖIER, K. 1954. Aspects of the agricultural value of certain barley X-ray mutations produced and tested at the Swedish Seed Association, Svalöf, and its branch stations. Acta agr. scand. *4*: 515–543.

GUSTAFSSON, A. 1947. Mutations in agricultural plants. Hereditas, Lund. *33*: 1–100.

——— 1954a. Swedish mutation work in plants: Background and present organization. Acta agr. scand. *4*: 361–364.

——— 1954b. Mutations, viability, and population structure. Acta agr. scand. *4*: 601–632.

GUSTAFSSON, A., and O. TEDIN. 1954. Plant-breeding and mutations. Acta agr. scand. *4*: 633–639.

HOFFMANN, W. 1951. Ergebnisse der Mutationszüchtung. Vorträge über Pflanzenzüchtung 1951, herausgeg. vom Land- und Forstwirtschaftl. Forschungsrat eV. Bonn, pp. 36–53.

KELNER, A. 1948. A method for investigating large microbial populations for antibiotic activity. J. Bact., Balt. *56*: 157–162.

——— 1948. Mutation in *streptomyces flaveolus* induced by X-rays and ultraviolet light. J. Bact., Balt. *56*: 457–465.

KUCKUCK, H. 1934. Züchterische und genetische Versuche mit Gerste. Naturwissenschaften *22*: 276–278.

MACKEY, J. 1954. Mutation breeding in polyploid cereals. Acta agr. scand. *4*: 549–557.

MULLER, H. J. 1927. Artificial transmutation of the gene. Science *66*: 84–87.

——— 1928. The problem of genic modification. Verh. V Internat. Kongr. Vererb. Berlin, 1927, *1*: 234–260 (Zsch. indukt. Abstamm., Supplementbd. 1).

NILSSON-EHLE, H. 1939. Polymere Röntgenmutationen bei *Hordeum*. Nova Acta Leop. N.F. *6*: 569–570.

NOVER, I. 1957. Sechsjährige Beobachtungen über die physiologische Spezialisierung des echten Mehltaus (Erysiphe graminis DC.) von Weizen und Gerste in Deutschland. Phytopath. Zsch. *31*: 85–107.

NOVER, I., und G. BANDLOW. 1958. Mutationsversuche an Kulturpflanzen. VIII. Mehltauresistenz und ihre Genetik bei Wintergersten-mutanten. Züchter *28*: 184–189.

NYBOM, N. 1954. Karyotype and viability in barley. Acta agr. scand. *4*: 507–514.

——— 1956. On the differential action of mutagenic agents. Hereditas, Lund. *42*: 211–217.

PROKOFIEVA-BELGOVSKAYA, A. A., S. I. ALIKHANIAN, O. N. KAPITONOVA, and E. I. EROKHINA. 1958. The cytology of the radiation mutants of *Actinomyces*. Izvest. Akad. Nauk SSSR, Ser. Biol. (Bull. Acad. Sc. USSR, Biol. Ser.), 1958, pp. 193–201 (Russian with English summary).

SAPEHIN, A. A. 1930. Röntgen-mutationen beim Weizen (*Triticum vulgare*). Züchter *2*: 257–259.

——— 1935. X-ray mutants in durum wheat. Bot. Z. USSR. *20*: 3–9 (Russian).

——— 1936. Röntgenmutationen bei Weichweizen. Trudy prikl. Bot. i pr. II, Genet., Plant Breed. Cytol. no. 9, 3 (1936) (Russian with English summary).

SEARS, E. R. 1956. The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Sympos. Biol. *9*: 1–22.

SCHOLZ, F. 1955. Mutationsversuche an Kulturpflanzen. IV. Ueber den züchterischen Wert zweier röntgeninduzierter nacktkörniger Gerstenmutanten. Kulturpflanze *3*: 69–89.

——— 1956. Mutationsversuche an Kulturpflanzen. V. Die Vererbung zweier sich variabel manifestierender Uebergangsmerkmale von bespelzter zu nackter Gerste bei röntgeninduzierten Mutanten. Kulturpflanze *4*: 228–246.

——— 1957. Mutationsversuche an Kulturpflanzen. VII. Untersuchungen über den züchterischen Wert röntgeninduzierter Mutanten verschiedener Merkmalsgruppen bei Sommer- und Wintergerste. 1. u. 2. Teil. Zschr. Pflanzenzücht. *38*: 181–220, 225–274.

——— 1958. Smooth-awned barley mutants induced by X-rays. Proc. X Internat. Congr. Genet. (in press).

——— 1958. Mutationsversuche an Kulturpflanzen. IX. Ueber den Rohproteingehalt röntgeninduzierter Gerstenmutanten. Züchter *28*: 283–296.

SCHOLZ, F., und CHR. O. LEHMANN. 1958. Die Gaterslebener Mutanten der Saatgerste in Beziehung zur Formenmannigfaltigkeit der Art *Hordeum vulgare* L.s.l. I. Kulturpflanze *6*: 123–166.

SCHREIBER, K. 1957. Natürliche pflanzliche Resistenzstoffe gegen den Kartoffelkäfer und ihr möglicher Wirkungsmechanismus. Züchter *27*: 289–299.

——— 1957. Neuere Untersuchungen auf dem Gebiet der Solanum-Alkaloide. Abh. Deut. Akad. Wiss., Berlin, Kl. Chem., Geol. Biol. 1956, *7*: 143–157.

——— 1958. Ueber einige Inhaltsstoffe der Solanaceen und ihre Bedeutung für die Kartoffelkäferresistenz. Ent. exp. & appl. *1*: 28–37.

STADLER, L. J. 1928. Mutations in barley induced by X-rays and radium. Science *68*: 186–187.

——— 1930. Some genetic effects of X-rays in plants. J. Hered. *21*: 3–19.

STUBBE, H. 1957. Mutanten der Kulturtomate *Lycopersicon esculentum* Miller. I. Kulturpflanze *5*: 190–220.

——— 1958. Mutanten der Kulturtomate *Lycopersicon esculentum* Miller, II. Kulturpflanze *6*: 89–115.

STUBBE, H., und G. BANDLOW. 1947. Mutationsversuche an Kulturpflanzen. I. Röntgenbestrahlungen von Winter- und Sommergersten. Züchter *17/18*: 365–374.

ZACHARIAS, M. 1956. Mutationsversuche an Kulturpflanzen. VI. Röntgenbestrahlungen der Sojabohne (Glycine soja (L.) Sieb. et Zucc.). Züchter *26*: 321–338.

MUTATIONS AT SPECIFIC LOCI IN *NEUROSPORA*[1]

Norman H. Giles[2]

THE SIGNIFICANCE for modern genetics of detailed studies of mutations at specific loci has become increasingly evident in the past few years. Historically, particular emphasis was placed on such studies by Stadler who performed classical experiments on mutation at the *R* and *A* loci in *Zea mays* (Stadler, 1954). More recently, increasing use has been made of various micro-organisms such as molds, bacteria, and bacteriophages. Numerous investigators have shown that these organisms possess many advantages for understanding both the mechanism of mutation and the organization and action of the genetic material.

This discussion will deal with recent results obtained in a continuing investigation of mutations at specific loci in *Neurospora crassa* (Giles, 1951, 1956). Ever since the historic experiments of Beadle and Tatum (1941), who produced the first biochemical mutants in this organism, *Neurospora* has become increasingly useful in genetic investigations. Cytogenetic studies have established seven well-marked linkage groups (McClintock, 1945; Singleton, 1953; Houlahan *et al.*, 1949; Barratt *et al.*, 1954); newly developed techniques make it feasible to obtain quantitative data on the rates of both forward and reverse mutation at specific loci and greatly facilitate the isolation of such mutants (Woodward *et al.*, 1954; Giles, 1951, 1956); biochemical investigations have identified the individual reactions blocked in numerous groups of allelic mutants and enzymatic evidence is now available for several of these groups (Horowitz and Fling, 1956); finally, the availability of tetrad analysis greatly facilitates genetic studies of individual mutants and permits investigations of recombination mechanisms in interallelic crosses which would otherwise be impossible (Mitchell, 1955; Case and Giles, 1958a, b).

In this paper, principal attention will be devoted to mutations at the *ad-4* locus. This locus is especially favorable, both biochemically and genetically, since the biochemical consequences of mutation at the locus are now well established and the locus is also well characterized genetically.

Before discussing the results of these studies, I would like to express my indebtedness to my present and former research colleagues and students at Yale who have participated directly or indirectly in these experiments: in particular, Drs. Mary Case, Fred deSerres, Norma Nelson, and Chester Partridge; Messrs. Tatsuo Ishikawa, Brooke Webber, and Dow Woodward.

EXPERIMENTAL MATERIALS AND METHODS

The initial studies to be reported have involved a total of thirty-five primary *ad-4* mutants (also referred to as F mutants), all but two having arisen by forward (direct) mutation in wild-type strain 74A (or closely related strains). Details on

[1]The research reported in this paper has been supported in part under contract AT (30-1)-872 with the Atomic Energy Commission.

[2]Department of Botany, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Conn., U.S.A.

the origin of these mutants, all of which were recovered in filtration-concentration experiments, are given in Table I.

Heterocaryon and crossing tests have shown that the thirty-three new primary mutants obtained in these studies are biochemically and genetically similar to the two previously known adenine-specific mutants detected in the early experiments of Beadle and Tatum (1945) and designated as *ad*-4 mutants by Barratt *et al.* (1954).

TABLE I

CLASSIFICATION OF PRIMARY MUTANTS AT THE *ad-4* LOCUS

Parental wild-type strain	Mutagen*	Mutants
74A	X-ray	F_1–F_6
		F_8, F_9
		F_{11}–F_{14}
		F_{20}, F_{21}
		F_{25}–F_{29}
	Ultraviolet	F_{19}
		F_{30}–F_{35}
	None	F_7†, F_{10}
5.5A	X-ray	F_{16}–F_{18}
	None	F_{23}
3.1a	None	F_{24}
Abb4A	Ultraviolet	F_{15}(= 44206t)‡
		F_{22}(= 44415t)‡

*X-ray dose = 36,000 r except for F_5 and F_6 where dose was 10,000 r; ultraviolet dose = 6,000 ergs/mm.^{2} for mutants 19, 30, 34, 35; 3000 ergs/mm.^{2} for mutants 31–33.

†In Table 1 of Giles, Partridge, and Nelson (1957), this mutant is incorrectly recorded as F_6.

‡Mutants obtained in experiments of Beadle and Tatum (1945).

Previous biochemical investigations with *ad-4* mutants established that these adenine-specific strains are blocked in the terminal reaction in adenylic acid biosynthesis —the splitting of adenosine monophosphate succinate (AMPS) to adenosine monophosphate (AMP)—and lack or have impaired activity for the enzyme adenylosuccinase (Giles, Partridge, and Nelson, 1956; 1957; Partridge and Giles, 1957). These experiments also revealed that F mutants lack enzymatic activity for a second reaction in adenine biosynthesis—the splitting of 5-amino-4-imidazole-(N-succinylo-carboxamide) ribotide (SAICAR) to 5-amino-4-imidazole carboxamide ribotide (AICAR) (Buchanan, 1957). Initial studies of filtrate accumulation products of a presumptive double adenine mutant (EF) (Giles *et al.*, 1957) appeared to require a revision of the initially proposed relations of the two groups of adenine-specific mutants—E and F (Giles *et al.*, 1956). However, subsequent genetic studies have shown that the presumptive EF double mutant, although obtained by tetrad analysis from a E × F cross, was actually an E mutant, presumably having arisen after isolation as a result of reversion at the F locus. Further biochemical studies with newly derived, stable EF doubles (identified by both heterocaryon analysis and outcrossing) have demonstrated that such strains do not accumulate hypoxanthine. Rather, when grown on limiting levels of adenine their culture filtrates have an absorption spectrum essentially similar to that for SAICAR. Coupled with the enzyme data, these results indicate that *ad-4* mutants are blocked in not one but two sequential, non-adjacent reactions in adenylic acid biosynthesis —the splitting of SAICAR to AICAR and of AMPS to AMP (Fig. 1). To date

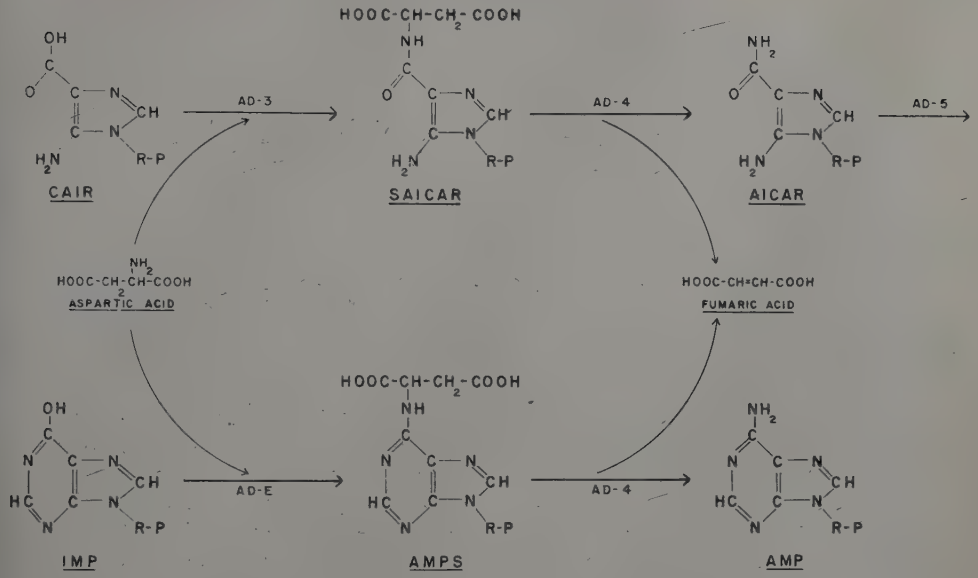


FIGURE 1. Final reactions in the biosynthesis of adenylic acid (AMP) in *Neurospora crassa* emphasizing similarities in the formation and splittng of SAICAR and AICAR. Positions of known mutant blocks are indicated.

these two activities have not been separable during enzyme purification procedures (Partridge, unpublished). These and other biochemical and genetical data strongly support the view that individual mutations at the *ad-4* locus lead to altered activity of a single enzyme—adenylosuccinase—which catalyses two separate but chemically related reactions (cf. Gots, 1957). No evidence exists for the participation of any other locus in the control of this enzyme.

Detailed linkage studies with mutants F_1 through F_{21} (Nelson, 1957) have established the location of all these F mutants at the *ad-4* locus in linkage group III between the two loci *pro-1* and *leu-1*. Ascospore platings, made according to the method developed by Newmeyer (1954), indicate, in general, a considerable homogeneity in the linkage values for the various mutants, and place the *ad-4* locus approximately eight crossover units distal to the *pro-1* locus and two crossover units proximal to the *leu-1* locus. In certain mutants, however (for example, F_{13} and F_{14}, and possibly others), the linkage data suggest the presence of chromosomal rearrangements.

Further extensive genetic studies have shown that crosses between most *ad-4* mutants give a low frequency of adenine prototrophs, whereas control selfings do not. These results indicate the existence of numerous mutational sites within the *ad-4* locus, although the data are not yet sufficient to establish a satisfactory genetic map of the locus because of the occurrence in most crosses of poor ascospore fertility. In these interallelic crosses the behavior of adjacent closely linked markers on either side of the locus indicates the existence of a strong correlation effect

(Freese, 1957) associated with the origin of adenine prototrophs. Although extensive tetrad data have not yet been obtained at this locus, these results from random ascospore platings, by analogy with those obtained at the *pdx-1* locus (Mitchell, 1955) and at the *pan-2* locus (Case and Giles, 1958a, b), furnish strong evidence for the occurrence in these crosses of the phenomenon referred to as "gene conversion." This situation thus provides an unusual opportunity for comparing the enzymatic activities of two types of "reversions"—those arising in conidia and those arising at meiosis. As will appear later, such comparisons indicate that the mechanisms of "reversion" are not the same in the two different situations.

A final feature of major significance in characterizing mutants at the *ad-4* locus is the recently discovered phenomenon of interallelic complementation. This type of complementation in *Neurospora* may be defined as the manifestation of a particular function by a heterocaryon between allelic—isolocal (Freese, 1957)—mutants, each of which lacks this function. In this instance, complementation between certain *ad-4* alleles results in the restoration of the adenylosuccinase activity which is absent in the individual mutants. No evidence exists for the occurrence of nuclear recombination in such heterocaryons. Hence, complementation appears to arise from some type of nuclear product interaction occurring in the cytoplasm (Giles, Partridge, and Nelson, 1956, 1957). Further studies of this phenomenon with larger numbers of *ad-4* mutants (Woodward *et al.*, 1958) indicate that the pattern of complementation exhibited by these mutants can be described in terms of a linear *complementation map*. In addition to its obvious significance for any interpretation of gene action, especially in relation to enzyme formation, such a complementation map provides additional criteria by which mutational changes at this locus can be characterized.

Reverse Mutation Studies

One of the first problems investigated with *ad-4* mutants was the nature of reversions to adenine independence since these mutants provide exceptional opportunities to characterize reversions in both genetic and biochemical terms. Forward mutation at this locus gives mutants requiring adenine and lacking or having impaired activity for a single enzyme—adenylosuccinase. The question may then be asked whether such mutants can undergo reversion to adenine independence and if so what genetic and biochemical mechanisms are responsible for these changes.

Tests of twenty-three out of the first twenty-four F mutants (all except F_{22}), the majority of which were derived from conidia exposed to X-rays, have indicated that all but four are capable of reversion, either spontaneously or following exposure to X-rays or ultraviolet (Nelson, 1957). These tests also demonstrate that individual mutants differ markedly in their reversion rates and that a particular allele has a certain intrinsic rate. Such *ad-4* mutants may be designated mutability isoalleles as has been done with other isolocal mutants in *Neurospora* (Giles, 1951, 1956).

Detailed biochemical and genetic studies have been made of reversions (recovered in mutant stocks carrying markers to detect possible contaminants) in only a limited number of *ad-4* mutants, but the results have already revealed interesting features of the reversion process. All reversions tested have been found to have restored adenylosuccinase activity for the AMPS substrate, which has been used in all assays unless otherwise noted. Activity assays are carried out by following the change in absorption at 280 mμ on a recording spectrophotometer (Giles *et al.*, 1957). Activities in different reversions range from approximately 3 per cent to 150 per cent of that in the parental wild type. Crosses employing linked markers

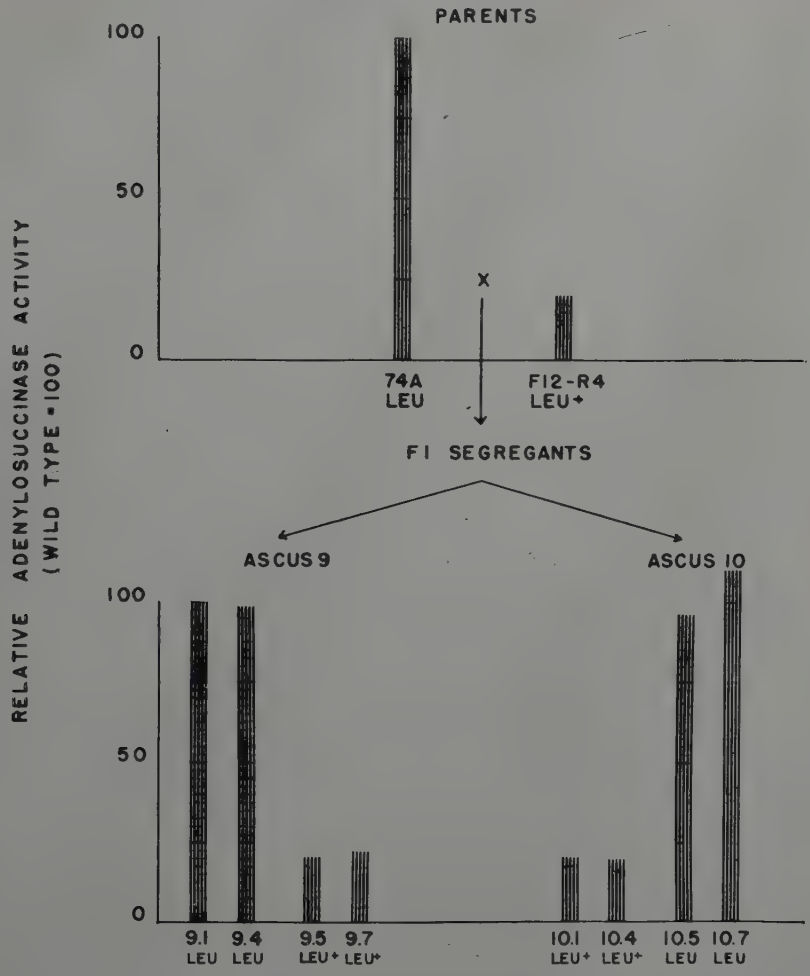


FIGURE 2. Evidence for regular tetrad segregation of allele-specific differences in adenylosuccinase activity. Reversion F_{12}-R_4 (derived from *ad-4* mutant F_{12}) has been crossed with wild type 74A carrying the *leu-1* marker which is closely linked with the *ad-4* locus.

indicate that these quantitative differences in adenylosuccinase activities characteristic of individual revertants are inherited as allelic differences at the *ad-4* locus. Figure 2 gives representative tetrad data from a cross between a wild type carrying the linked *leu-1* marker and a reversion having an adenylosuccinase activity approximately 25 per cent of that of the wild type. These results indicate a regular 1:1 segregation for the two parental types, the leucine-requiring segregants having the high activities characteristic of the parental wild type with the leucine marker. Additional tests have utilized revertants exhibiting other enzyme activity levels crossed with wild type, as well as crosses of two different revertant types (for example, ones with activities of approximately 3 per cent and 25 per cent respectively). Although a certain amount of variation in activity compared with the parental strains is encountered in segregants, this rarely exceeds 20 per cent and in every instance

classification of the segregating types has been clear-cut. The present data thus suggest that modifiers at other loci are of relatively minor importance in determining the levels of adenylosuccinase activity in various revertants.

Results of the type just discussed provide evidence that reversions in F mutants arise from reverse mutational changes at the *ad-4* locus rather than from suppressor mutations at other loci. More extensive tests of this point have been made by back-crossing homocaryotic reversions to wild type and testing ascospores isolated at random for the occurrence of adenine-requiring (*ad-4*) recombinants.

Tests of a large number of revertants in one mutant (F_{12}) involved preliminary screenings to detect either unlinked or loosely linked suppressors. In all, crosses of 34 ultraviolet-induced homocaryotic revertants of independent origin were made. From each cross 100 ascospores were isolated and the resulting colonies tested on minimal. The number of colonies tested from these crosses ranged from 54 to 96, the average being 81. No adenine-requiring segregants were recovered. To detect possible closely linked suppressors, more extensive tests have been performed with two of the above ultraviolet-induced revertants from F_{12} and with additional revertants produced by various mutagenic treatments in F_{12} and in other primary and secondary *ad-4* mutants. In these tests, general isolation and testing of individual colonies have not been attempted, since later studies developed procedures suitable for the visual detection of occasional adenine-requiring F-mutant colonies in ascospore platings yielding large numbers of adenine-independent colonies. Tests of these methods by reconstruction and forward mutation experiments indicate that 90 per cent of any *ad-4* mutants present should be detectable by the procedures employed. In individual crosses, any questionable colonies have been isolated and tested as potential *ad-4* segregants. The results of such tests are indicated in Table II. Although as many as 14,000 colonies from certain crosses have been examined in these platings, no adenine-requiring segregants have been recovered.

TABLE II

TESTS FOR POSSIBLE SUPPRESSORS IN VARIOUS *ad-4* REVERSIONS*

Reversion	Mutagen	Adenylosuccinase activity category (as percentage of wild type)	Viable ascospores plated	Adenine-requiring isolates
F_{12}–R_{19}	X-ray	25	8,323	0
F_{12}–R_{164}	Ultraviolet	25	7,269	0
F_{12}–R_{165}	Ultraviolet	25	7,057	0
F_{12}–R_{13}	X-ray	3	11,873	0
F_{12}–R_{28}	X-ray	3	6,348	0
F_4–R_{23}	X-ray	100	6,267	0
F_4–R_{24}	X-ray	150	8,330	0
F_4–R_{27}	X-ray	100	1,424	0
F_{23}–R_4	X-ray	25	2,163	0
F_{23}–R_5	X-ray	25	9,483	0
F_{23}–R_9	X-ray	65	3,472	0
F_{19}–R_{201}	Ultraviolet	—	9,178	0
F_{19}–R_{203}	Ultraviolet	—	9,501	0
F_{12}–R_{28}–M_8–R_{36}†	X-ray	3	1,422	0
F_{12}–R_{28}–M_{14}–R_1†	X-ray	3	14,133	0

*All reversions were crossed with wild type. For further details, see text.
†Secondary revertants (cf. Figure 4).

Further tests have involved the use of filtration-concentration procedures employing ascospores from crosses of various revertants with wild type. Again no *ad-4* segregants have been recovered. Although difficulties are encountered in these experiments in making accurate estimates of the number of viable ascospores tested and of the efficiency of mutant recovery, present estimates are that over 10^6 viable ascospores from various crosses have been screened by this procedure. These results

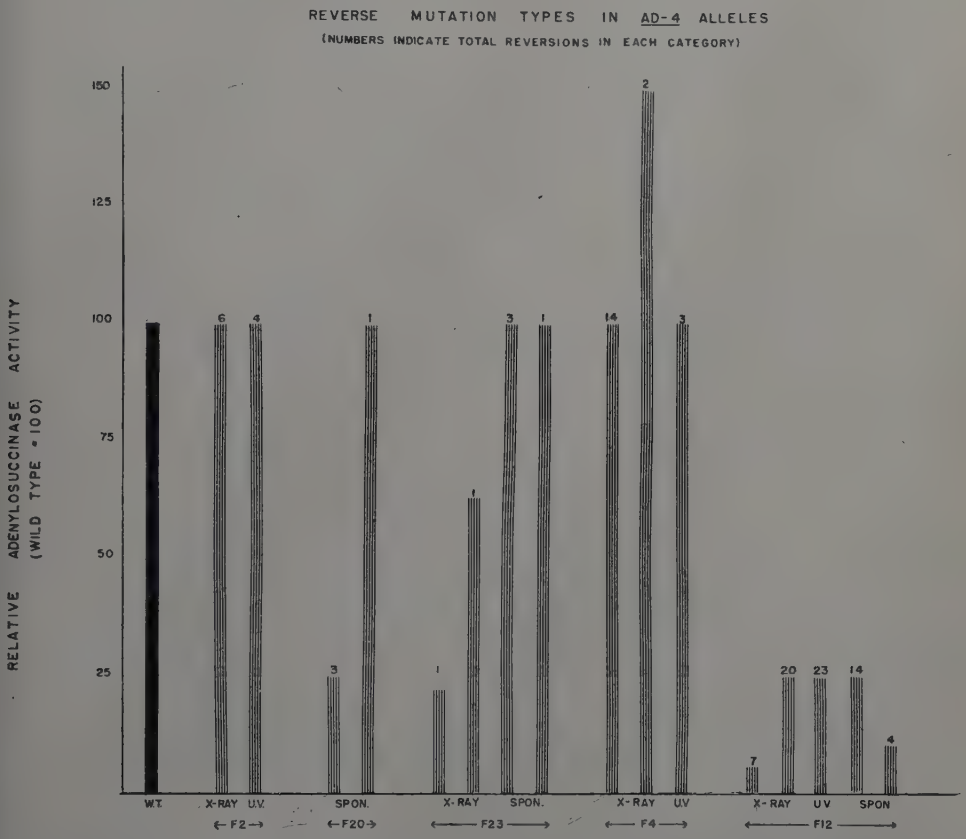


FIGURE 3. Categories of reverse mutation types with respect to adenylosuccinase activity occurring in various *ad-4* alleles. The diagram indicates the five alleles studied, the origin of the reverse mutants, and the number of reversions in each category.

provide further evidence that these reverse mutations are indistinguishable from wild type on the basis of recombination tests. Since the frequency of wild-type recombinants in crosses of different *ad-4* alleles ranges from 1/500 to 1/50,000 viable ascospores, the present data make it unlikely that suppressor mutations even within the *ad-4* locus have produced the reversions tested in these crosses.

Of particular interest has been the finding, based on quantitative adenylosuccinase assays of a large series of reversions, that there appears to be a limited number of discrete reverse mutation categories. Furthermore, each *ad-4* mutant studied to date exhibits a distinct pattern of reverse mutation with respect to these categories. These results are summarized in Figure 3. (These assays have been made on mycelial

extracts prepared in tris buffer, which gives more stable, but sometimes lower activities than the phosphate buffer previously employed (Giles *et al.*, 1957).) Certain mutants can undergo reverse mutation to yield strains with activities equivalent to, or in F_4 even surpassing that of the parental wild-type strain. Mutant F_{12}, however, has never given a reversion equivalent to wild type in enzyme activity. This restriction appears to be characteristic of this particular allele, and is not an effect of the mutagen employed in producing reversions, since no reversions tested, whether of X-ray, ultraviolet, or spontaneous origin, have had activities greater than approximately 25 per cent of wild type.

Additional evidence concerning mutational phenomena at the *ad-4* locus has been sought by experiments involving the production of successive alternate forward

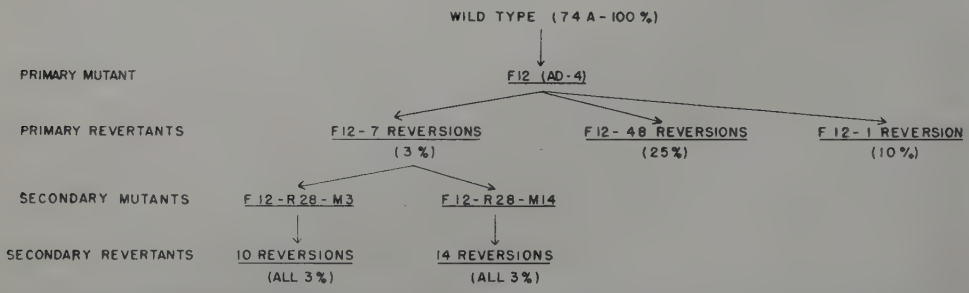


FIGURE 4. The production of successive alternate forward and reverse mutations, employing the *ad-4* mutant F_{12}. Percentages indicate adenylosuccinase activity relative to wild type 74A.

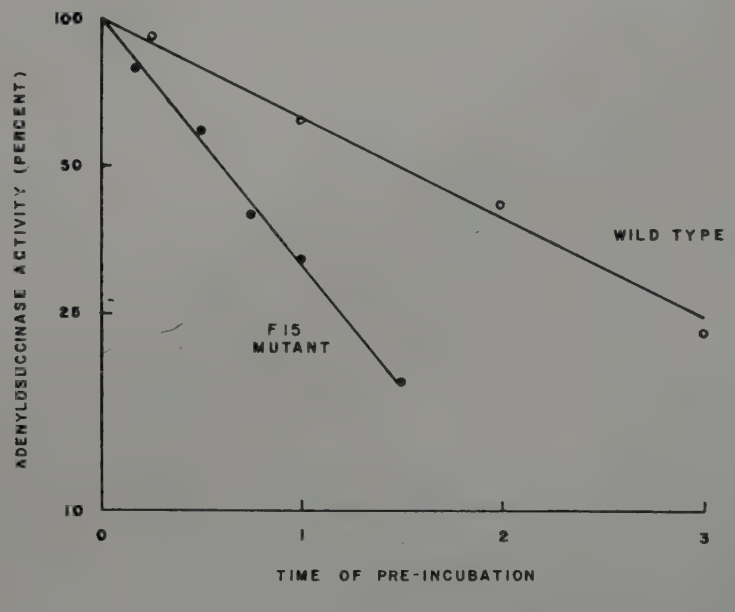


FIGURE 5. Evidence for a thermolabile adenylosuccinase in the temperature-sensitive mutant F_{15}. The comparison involves the relative loss in enzyme activity following pre-incubation at 48° of crude mycelial extracts from wild type (74A) and mutant F_{15}.

and reverse mutations, employing the F_{12} mutant. These experiments provide further evidence that certain mutational changes may place permanent upper limits on the degree to which the *ad-4* locus can be restored either spontaneously or by further physical treatment. A summary of these studies is presented in Figure 4. The results indicate that, just as primary mutant F_{12} is apparently unable to yield primary revertants with enzyme activities above approximately 25 per cent, a primary revertant (F_{12}-R_{28}) with a 3 per cent enzyme activity level yields secondary mutants (F_{12}-R_{28}-M_3 and M_{14}) which are apparently incapable of yielding secondary revertants with enzyme activities above 3 per cent. Tests have not yet been made to determine whether a similar mutational behavior is characteristic of primary revertants of F_{12} in the 10 and 25 per cent categories, as well as of particular revertant types derived from other F mutants such as F_{20} and F_{23}.

In addition to quantitative distinctions among *ad-4* alleles, certain alleles can also be distinguished in terms of qualitative differences in the enzymes produced in their presence. For example, one of the temperature-sensitive forward mutants (F_{15}), which grows on minimal at 25°C. but not at 35°C., makes an adenylosuccinase which is considerably more thermolabile than is that in wild type (Fig. 5). Additional general evidence has been obtained for qualitative distinctions among the enzymes produced by varous revertants. These enzymatic tests have involved observations on relative inhibition by metals, and on various factors influencing activation or inactivation (Partridge, unpublished).

Relative Substrate Specificities

Ad-4 alleles are of particular interest in that they provide an opportunity to test for possible enzymatic differences in mutants and revertants with respect to relative substrate specificities utilizing the two natural substrates AMPS and SAICAR. As indicated previously (Fig. 1), all the available evidence supports the hypothesis that mutation at the *ad-4* locus leads to altered activity of a single bifunctional enzyme. Indeed, a cogent part of this evidence comes from experiments with forward as well as with reverse mutants (Fig. 6). Initial results with all mutants tested demonstrated a marked correspondence in their relative activities for AMPS and SAICAR. However, recent preliminary tests suggest that allelic distinctions with respect to activities for the two substrates may exist. Comparative activity tests have been made with a revertant (F_{12}-R_4) induced in mutant F_{12}, together with segregating isolates from crosses of this revertant to both wild type (carrying the linked *leu-1* marker) and the parental F_{12} mutant. The results (Fig. 7) indicate that in comparison with wild-type enzyme, the enzyme from the revertant is markedly less active for SAICAR than for AMPS. Although variations in activities, especially for SAICAR, are encountered in the segregating wild-type isolates, and to some extent in the mutant isolates, the general segregation pattern again indicates a relatively greater activity for AMPS in the revertant segregants, thus supporting the hypothesis that this distinction is inherited and allele-specific.

These preliminary observations have been confirmed by additional very recent assays on highly purified preparations of SAICAR. Additional experiments are underway both to purify the enzyme from the F_{12}-R_4 revertant, so as to facilitate further comparative activity studies, and to isolate selectively revertants which may exhibit even greater substrate specificity differences—as, for example, a complete absence of activity for one of the two substrates.

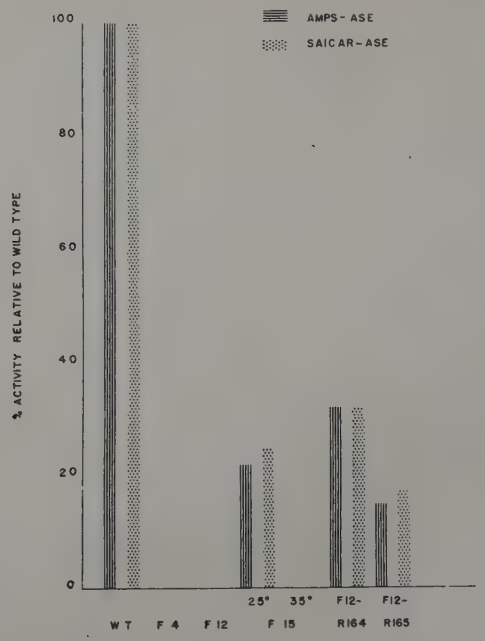


FIGURE 6. Relative enzyme activities (compared with wild type) for the two natural substrates AMPS and SAICAR exhibited by preparations from several *ad-4* mutants and revertants. Assays made on extracts prepared in phosphate buffer (see text).

GENE CONVERSION STUDIES

As indicated previously, crossing evidence has shown that numerous mutational sites exist within the *ad-4* locus. The results of random ascospore isolations from various *ad-4* mutant crosses employing closely linked markers on either side of the locus indicate the existence of a strong correlation effect, such that although many adenine prototrophs occur in one recombinant class with respect to the adjacent markers, the two parental (non-crossover) and the reciprocal crossover classes are almost always present with relatively high frequencies. These results, which are similar to those reported recently at numerous other loci in *Neurospora* (Freese, 1957), *Aspergillus* (Pontecorvo, 1956), and other organisms, are unexpected on the basis of conventional crossover mechanisms. Although definitive tetrad data indicating atypical segregation have not yet been obtained for *ad-4* mutant crosses, the present results strongly support the hypothesis that recombination at this locus involves novel mechanisms for which terms such as "gene conversion" and "copy-choice" (Lederberg, 1955) have been employed. The gene conversion hypothesis, as originally proposed (Lindegren, 1953), interprets atypical segregations on the basis of directed mutations occurring at meiosis in a heterozygote such that one allele is "converted" into another, whereas the copy-choice hypothesis (Lederberg, 1955) interprets atypical segregations essentially in terms of replication errors such that one of the two newly formed chromatids in a tetrad replicates using its homologue as a template.

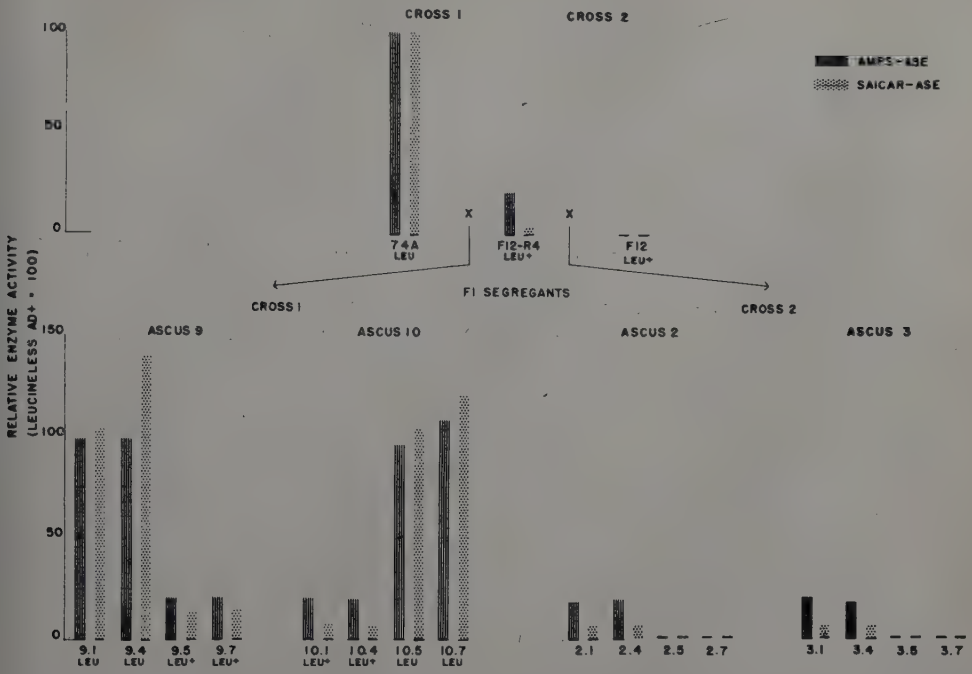


FIGURE 7. Evidence that enzyme preparations from reverse mutant F_{12}-R_4 differ in their relative activities for the two natural substrates AMPS and SAICAR and that this difference is allele-specific. Crosses of F_{12}-R_4 for tetrad analysis have been made with wild type 74A carrying the *leu-1* marker (linked to the *ad-4* locus) and with *ad-4* mutant F_{12} with no marker.

The *ad-4* locus provides an unusual opportunity to test certain aspects of these two different hypotheses. From crosses of various *ad-4* alleles, selective isolation procedures can be used to obtain adenine prototrophs of meiotic origin carrying parental rather than crossover markers, and hence presumably not the result of conventional reciprocal crossover events. Enzyme assays can then be made of these so-called meiotic "reversions" and these results compared with similar assay data from reversions arising in conidia of the same alleles. Of particular interest have been the results with reversions derived from reciprocal crosses (with respect to the two linked markers, *pro-1* and *leu-1*) between F_{10}, a stable mutant which has never yielded conidial reversions, and F_{12}, the mutant which has never yielded conidial reversions with activities above 25 per cent of wild type. In tests of twenty reversions from this cross, all carrying the *leu-1* parental marker and hence presumably not the result of conventional reciprocal crossover events, most had activities equivalent to wild type and none had activities in the low categories characteristic of conidial reversions of F_{12}. Similar tests with a variety of other *ad-4* alleles involving crosses of the type illustrated in Figure 8 have given results generally comparable to those just discussed, as shown in Figure 9.

The over-all comparative data on enzyme assays for the two classes of "reversions" —those from conidia and those from interallelic crosses—strongly support the view that the mechanisms by which "reversions" arise at meiosis are basically different from those by which reverse mutations arise in conidia and to this extent do not support the gene conversion hypothesis as originally proposed. Furthermore, the

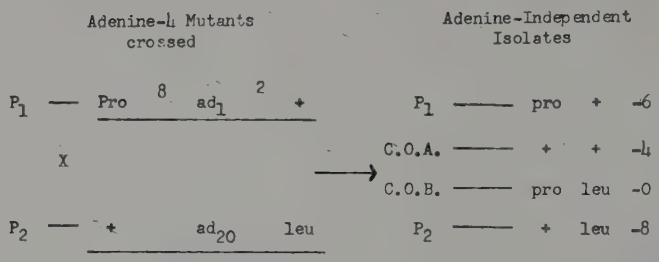


FIGURE 8. Procedure used to obtain prototrophs from crosses of *ad-4* alleles carrying linked markers. The adenylosuccinase assays shown in Figure 9 were made with isolates carrying the P_2 marker (*+leu*), most of which presumably arose by some type of gene-conversion mechanism.

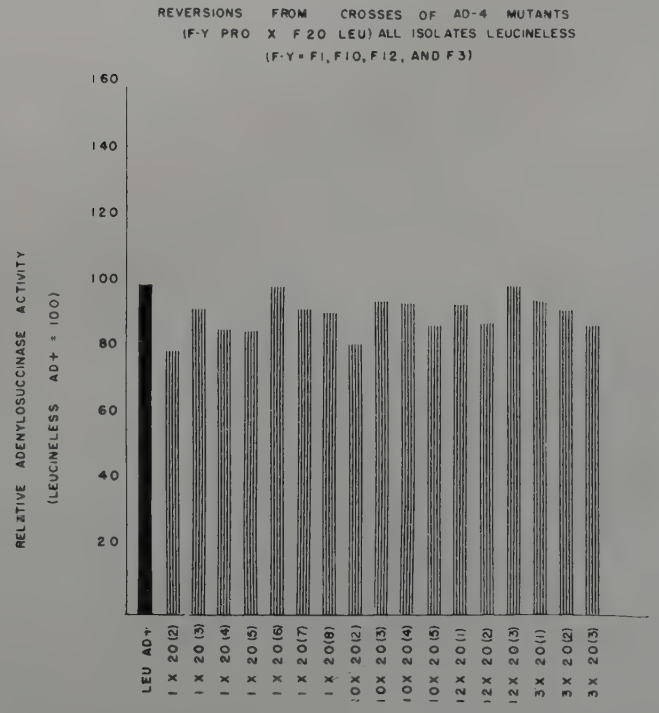


FIGURE 9. Adenylosuccinase assays of prototrophs derived from interallelic *ad-4* crosses of the type shown in Figure 8. These adenine-independent isolates thus represent "reversions" arising at meiosis in contrast to those arising in conidia by reverse mutation (cf. Fig. 3).

finding that the majority of reversions derived from meiosis are essentially equivalent to wild type in enzyme activities does support the hypothesis that some type of copy-choice event is responsible for the origin of all adenine prototrophs not arising by conventional crossing-over. Thus the present results agree with the conclusions derived from the extensive tetrad data at the *pan-2* locus (Case and Giles, 1958a,

b). In those studies the available criteria indicated that all isolates from tetrads exhibiting atypical mutant segregations (with no pan prototrophs present) were identical with either one or the other of the two parental *pan-2* mutants.

Interallelic Complementation Studies

As discussed previously, one of the unexpected results of the present series of studies with *ad-4* mutants was the discovery of complementation in heterocaryons between adenylosuccinaseless alleles resulting in the restoration of adenylosuccinase activity. These early observations indicated that the majority of the initially available *ad-4* alleles failed to complement at all. Although the few combinations which did complement clearly demonstrated the existence of distinct functional regions of some type at the *ad-4* locus, no clear relation of these units to one another was

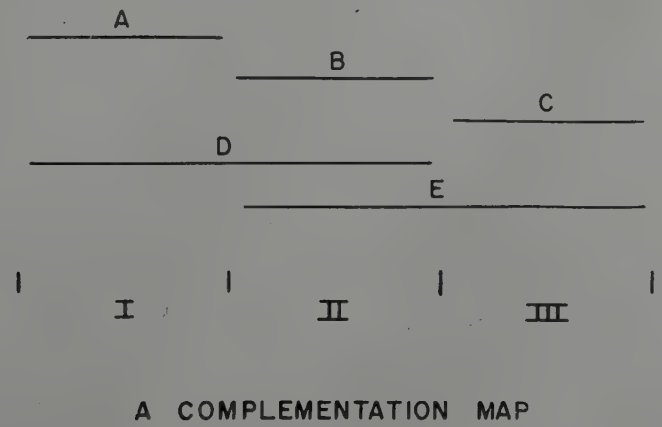


Figure 10. A hypothetical complementation map based on the heterocaryon responses of five allelic biochemical mutants. Non-overlapping mutant combinations exhibit complementation, while overlapping combinations do not. The existence of three distinct functional regions (cistrons) is indicated (Roman numerals I–III).

evident. Subsequent studies of this phenomenon by Mr. Dow Woodward, who has utilized much larger numbers of *ad-4* mutants of both primary and secondary origin, have demonstrated the existence of a striking pattern of complementation which now appears to be of widespread occurrence and presumably of basic genetic significance. The relations of the complementing mutants at the *ad-4* locus are such that these mutants can be arranged in a unique linear sequence which may be designated a complementation map of this locus. This arrangement is possible because of the apparent presence in certain alleles of continuous multiple non-functional regions which overlap non-functional regions of other alleles. The method employed in constructing such a complementation map is shown diagrammatically in Figure 10. In this hypothetical example, mutants A, B, and C each complement one another, indicating the existence of three distinct functional regions (cistrons), but providing no evidence for an ordered linear relationship. Mutants D and E, however, are multiple types such that D complements only C, and E only A. This relation permits the establishment of a unique linear arrangement with mutant B between mutants A and C.

The actual complementation map of the *ad-4* locus (Fig. 11) is much more complex than the example just given. Among the 42 (out of 51 available) complementing *ad-4* mutants tested, 16 different complementing types have been detected, whose relations provide evidence for seven distinguishable functional regions (cistrons) at this locus. The present map has been constructed from both primary *ad-4* mutants derived from wild-type and also from secondary *ad-4* mutants derived from

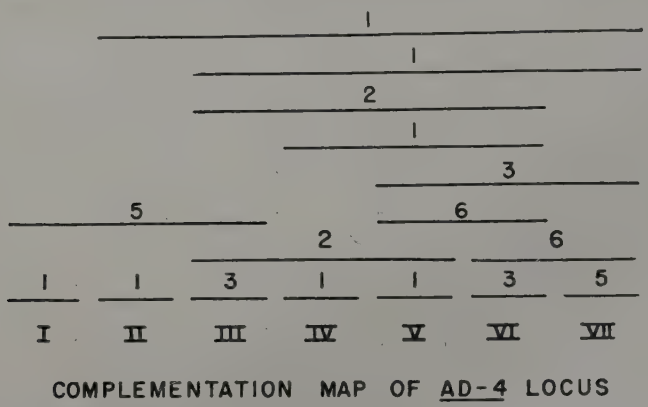


FIGURE 11. Complementation map of *ad-4* locus based on both primary and secondary mutants. Roman numerals indicate seven distinct functional regions (cistrons). Figures above complementation types indicate numbers of *ad-4* mutants in each category.

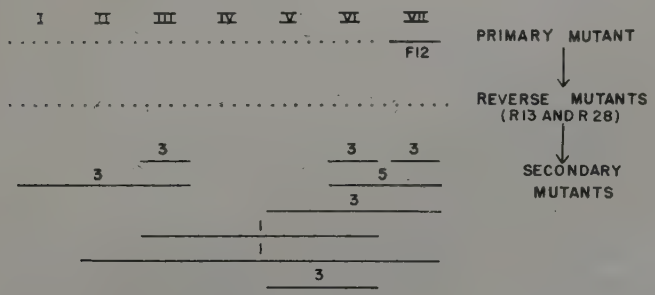


FIGURE 12. Complementation types found in secondary forward mutants produced by X-irradiation of two primary reverse mutants of F_{12} (R_{13} and R_{28}) of X-ray origin. Figures above complementation types indicate number of *ad-4* mutants in each category.

three different reversions—two derived from F_{12} and having an enzyme activity level 3 per cent of wild type, and one derived from F_2 and having an activity level equivalent to wild type. The point of major significance in this regard is that all mutants, whether of primary or secondary origin, can be located on a single complementation map.

The complementation relations of F_{12} itself, compared with those of secondary mutants derived from F_{12} revertants, can be described formally in terms of the repair of one functional region by reverse mutation followed by damage in other regions during subsequent forward mutation to produce secondary mutants, as

shown in Figure 12. There is, however, evidence that such an interpretation is too simple. Woodward *et al.* (1958) have now made extensive quantitative measurements of adenylosuccinase activities in complementing heterocaryons. Their results indicate that with primary mutant combinations (and in combinations of primary with secondary mutants) activities range from less than 1 per cent to 25 per cent of that found in the parental strain. In general, there is a striking direct correlation between the relative distance apart of any two mutants on the complementation map and the level of enzyme activity in a heterocaryon between them. Thus heterocaryons between mutants damaged in adjacent cistrons have low activities, and activities increase with increasing distance to the maximum value of 25 per cent for mutants separated by four to six cistrons. However, enzyme activities never exceed 3 per cent in complementing heterocaryons between secondary mutants derived from the two F_{12} revertants which themselves have enzyme activities of only 3 per cent. These results provide additional evidence that such reverse mutants are not equivalent to wild type. In addition, even though the F_2 revertant has an activity equivalent to wild type, preliminary quantitative data from its complementing heterocaryons also suggest that this revertant is not identical with the parental wild-type strain.

A major problem relative to the phenomenon of complementation is the existence of such large numbers of completely non-complementing mutants. These cannot be interpreted on the basis of multiple damage such as would result from large deletions, since many of these mutants readily undergo reverse mutation. Indeed, the reversion experiments indicate that a non-complementing primary mutant such as F_2 may yield complementing secondary mutants. Additional studies will be required to interpret the frequent non-complementing types.

The discovery of interallelic complementation at the *ad-4* locus, and in particular the construction of a complementation map of this locus, immediately raises a question as to the general significance of this phenomenon. Accumulating evidence indicates that complementation, and indeed complementation maps, are of widespread occurrence and may well prove to be of basic significance for an understanding of gene mutation and gene action.

In our studies at Yale, several additional instances of complementation have been examined, and at four of these loci complementation maps have been constructed. Preliminary maps of three loci are presented in Figure 13. Evidence for complementation in enzyme formation is being presented at these meetings by Fincham and by Lacy and Bonner, in addition to the report by Woodward. Several additional instances of complementation have been observed by Catcheside and Overton (1958) who have also constructed a complementation map of the *arg-2* locus. Complementation also occurs in micro-organisms other than *Neurospora,* for example, in *Aspergillus* (Calef, 1956) and also in *Salmonella,* where the data of Hartmann on abortive transductions (also presented at this meeting) provide evidence for a complementation map at the *hist-B* locus.

The discovery of a non-genetic method which establishes linear sequences of allelic mutants strongly suggests that such sequences may reflect the linear organization of genes and their products. Indeed, certain newly available data support this hypothesis. As yet only preliminary recombination data are available for the *ad-4* locus, since crosses of these mutants are, in general, highly infertile. Many instances of interallelic recombination have been detected, however, and the present results suggest that the genetic map of this locus may correspond generally to the complementation map. Much more satisfactory comparative evidence is now available at another locus—the *pan-2* locus—as a result of studies reported at this meeting by Dr. Mary Case of Yale. On biochemical evidence, *pan-2* mutants are all blocked in

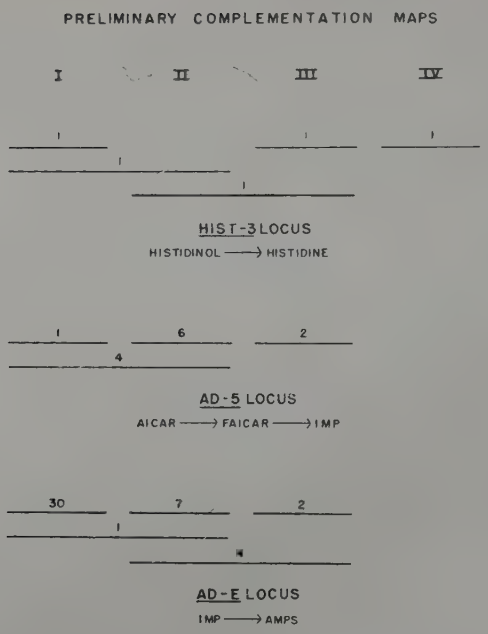


FIGURE 13. Preliminary complementation maps of three different loci in *Neurospora crassa*. The particular reactions blocked are indicated below each locus designation. Numbers indicate mutants of each complementing type.

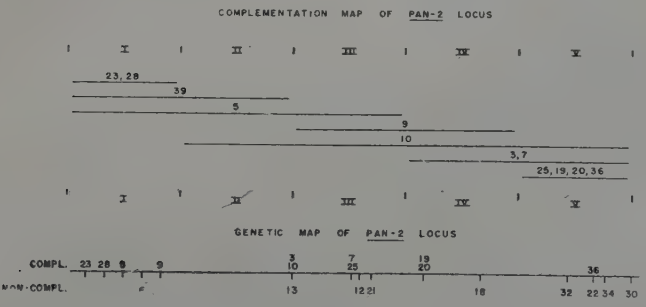


FIGURE 14. Comparative complementation and genetic maps of *pan-2* locus in *Neurospora crassa*. Numbers refer to individual *pan-2* mutants. On the genetic map, complementing mutants are indicated above the line, non-complementing ones, below.

the same reaction—the conversion of ketovaline to ketopantoate. Although enzymatic evidence has not yet been obtained in *Neurospora*, studies with *E. coli* mutants blocked in the same step in pantothenic acid biosynthesis have shown that a single enzyme catalyzes the reaction (Maas and Vogel, 1953). Dr. Case's comparative studies of some forty primary mutants provide clear evidence for a marked correspondence between the genetic and complementation maps of the *pan-2* locus, as shown in Figure 14.

Clearly, further extensive combined biochemical and genetic studies will be required to establish the significance of complementation maps and to interpret the mechanism of complementation in enzyme formation, even though a beginning has been made in the studies reported here and in those described by Fincham and others at this meeting. Since no evidence exists for nuclear recombination in these *Neurospora* heterocaryons, a reasonable working hypothesis at present appears to be that interallelic complementation involves the formation by different mutant nuclei in a heterocaryon of defective gene products which recombine in the cytoplasm, perhaps by a process analogous to crossing-over, at either the primary (RNA) or the secondary (polypeptide) level, such that enzymatically active protein is produced (Woodward *et al.*, 1958). Other interpretations are, of course, possible. However, the present hypothesis, when expressed in a more detailed form, is specially attractive, since it has certain qualitative and quantitative consequences which should make it subject to experimental testing.

Summary

This paper has described mutational studies at specific loci in *Neurospora*. Most of the discussion has dealt with results at the *ad-4* locus where combined genetic and enzymological experiments are feasible. This locus, which is in linkage group III between the *pro-1* and *leu-1* loci, controls the production of a single bifunctional enzyme—adenylosuccinase—required for the final step, as well as one earlier step in the biosynthesis of adenylic acid. No evidence exists for the participation of any other locus in the control of this enzyme. Mutational, recombinational, and functional evidence indicates that this locus is complex.

Forward (direct) mutations in wild type, which occur at numerous different mutational sites in the *ad-4* locus, lead to altered activity of the enzyme. Certain *ad-4* mutants differ in their abilities to undergo reverse mutation, not only in terms of frequencies, but also in terms of the maximum degree of restoration of enzyme activity in the resulting revertants. Reverse mutants fall into a few restricted groups, distinguishable by enzyme assay. Segregation data indicate that such differences in enzyme level are allele-specific. In addition to quantitative distinctions, evidence for other differences between certain alleles has been obtained in terms of qualitative distinctions between the enzymes produced in their presence, for example, in thermolability or in relative substrate specificities (with the use of the two natural substrates).

Genetic data from interallelic *ad-4* crosses provide evidence for the probable occurrence at meiosis of novel recombination mechanisms such as "gene conversion" or "copy-choice." Enzyme assay data on adenine prototrophs derived from such crosses (and carrying parental linked markers) indicate that as a group these strains are distinct from reverse mutants arising in conidia, and thus suggest that quite different mechanisms are involved in the origins of the two types of "reversions."

Further studies of the phenomenon of interallelic complementation in adenylosuccinase formation have led to the production of a complementation map of the *ad-4* locus on which complementing mutants can be arranged in a unique linear sequence. Quantitative adenylosuccinase assays of complementing heterocaryons have established a striking direct correlation between the relative distance apart of any two mutants on the complementation map and the level of enzyme activity in a heterocaryon between them.

The production of a number of successive alternate forward and reverse mutations at this locus has yielded both primary and secondary *ad-4* mutants. Experiments with these mutants indicate that the complementary relationships of certain mutants can be changed by further mutagenic treatment in two stages. These changes, when expressed as alterations in complementation map locations, correspond formally to repair of one functional region followed by damage in another.

Investigations at several other unrelated loci indicate that complementation and complementation maps are probably of widespread occurrence in *Neurospora*. Studies with *pan-2* mutants provide evidence for a marked correspondence in the arrangement of mutants on the independently derived genetic and complementation maps of this locus.

The over-all results of investigations on interallelic complementation promise to be of basic significance in elucidating the mechanisms by which gene products interact in enzyme formation.

REFERENCES

Barratt, R. W.; D. Newmeyer; D. D. Perkins; and L. Garnjobst. 1954. Map construction in *Neurospora crassa*. Advance. Genet. *6*: 1–93.

Beadle, G. W., and E. L. Tatum. 1941. Genetic control of biochemical reactions in *Neurospora*. Proc. Nat. Acad. Sc. U.S. *27*: 499–506.

——— 1945. *Neurospora*. II. Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Bot. *32*: 678–686.

Buchanan, J. M.; J. G. Flaks; S. C. Hartman; B. Levenberg; L. N. Lukens; and L. Warren. 1957. The enzymatic synthesis of inosinic acid *de novo*. *In* Ciba Foundation Symposium on the Chemistry and Biology of Purines, eds. G. E. W. Wolstenholme and C. M. O'Connor, pp. 233–255. London: Churchill.

Calef, E. 1956. Functional relationships between three adenineless mutants in *Aspergillus nidulans*. Heredity *10*: 279. (abstract)

Case, M. E., and N. H. Giles. 1958a. Evidence from tetrad analysis for both normal and aberrant recombination between allelic mutants in *Neurospora crassa*. Proc. Nat. Acad. Sc. U.S. *44*: 378–390.

——— 1958b. Recombination mechanisms at the *pan-2* locus in *Neurospora*. Sympos. Quant. Biol. *23*: 119–135.

Catcheside, D. G., and A. Overton. 1958. Complementation between alleles in heterocaryons. Sympos. Quant. Biol. *23*: 137–140.

Freese, E. 1957. The correlation effect for a histidine locus of *Neurospora crassa*. Genetics *42*: 671–684.

Giles, N. H. 1951. Studies on the mechanism of reversion in biochemical mutants of *Neurospora crassa*. Sympos. Quant. Biol. *16*: 283–313.

——— 1955. Forward and back mutation at specific loci in *Neurospora*. Brookhaven Sympos. Biol. *8*: 103–125.

Giles, N. H., C. W. H. Partridge, and N. J. Nelson. 1956. The genetic control of adenylosuccinase in *Neurospora crassa*. Proc. Internat. Genet. Sympos. Suppl. to Cytologia pp. 543–546.

——— 1957. The genetic control of adenylosuccinase in *Neurospora crassa*. Proc. Nat. Acad. Sc. U.S. *43*: 305–317.

Gots, J. S., and E. G. Gollub. 1957. Sequential blockade in adenine biosynthesis by genetic loss of an apparent bifunctional deacylase. Proc. Nat. Acad. Sc. U.S. *43*: 826–834.

Horowitz, N. H., and M. Fling. 1956. The role of the genes in the synthesis of enzymes. *In* Enzymes: Units of Biological Structure and Function, ed. O. H. Gaebler, pp. 139–145. New York: Academic Press.

Houlahan, M. B., G. W. Beadle, and H. G. Calhoun. 1949. Linkage studies with biochemical mutants of *Neurospora crassa*. Genetics *34*: 493–507.

LEDERBERG, J. 1955. Recombination mechanisms in bacteria. J. Cellul. Physiol. (Suppl. 2) *45*: 75–107.

LINDEGREN, C. C. 1953. Gene conversion in *Saccharomyces*. J. Genet., Cambr. *51*: 625–637.

MAAS, W. K., and H. J. VOGEL. 1953. Alpha-ketoisovaleric acid, a precursor of pantothenic acid in *Escherichia coli*. J. Bact., Balt. *65*: 388–393.

MCCLINTOCK, B. 1945. *Neurospora*. I. Preliminary observations of the chromosomes of *Neurospora crassa*. Am. J. Bot. *32*: 671–678.

MITCHELL, M. B. 1955. Aberrant recombination of pyridoxine mutants of *Neurospora*. Proc. Nat. Acad. Sc. U.S. *41*: 215–220.

NELSON, N. J. 1957. Genetical and biochemical investigations of adenine-specific mutants in *Neurospora crassa*. Unpublished thesis, Yale University.

NEWMEYER, D. 1954. A plating method for genetic analysis in *Neurospora*. Genetics *39*: 604–618.

PARTRIDGE, C. W. H., and N. H. GILES. 1957. Identification of major accumulation products of adenine-specific mutants of *Neurospora*. Arch. Biochem., N.Y. *67*: 237–258.

PONTECORVO, G. 1956. Allelism. Sympos. Quant. Biol. *21*: 171–174.

SINGLETON, J. R. 1953. Chromosome morphology and the chromosome cycle in the ascus of *Neurospora crassa*. Am. J. Bot. *40*: 124–144.

STADLER, L. J. 1954. The gene. Science *120*: 811–819.

WOODWARD, D. O., C. W. H. PARTRIDGE, and N. H. GILES. 1958. Complementation at the *ad-4* locus in *Neurospora crassa*. Proc. Nat. Acad. Sc. U.S. *44*: 1237–1244.

WOODWARD, V. W., J. R. DEZEEUW, and A. M. SRB. 1954. The separation and isolation of particular biochemical mutants of *Neurospora* by differential germination of conidia, followed by filtration and selective plating. Proc. Nat. Acad. Sc. U.S. *40*: 192–200.

POST-IRRADIATION METABOLISM AND THE TIMING OF ULTRAVIOLET-INDUCED MUTATIONS IN BACTERIA[1]

Evelyn M. Witkin[2]

IRRADIATION OF BACTERIA with ultraviolet light is followed by a sensitive period during which the genetic consequences of the exposure are modifiable. Induced mutations may be completely suppressed or their usual frequency multiplied more than a hundredfold by manipulation of conditions during the critical time. Despite the bewildering variety of post-treatments capable of changing the course of ultraviolet-initiated mutagenesis and the diversity of the pathways through which they exert their influence, there is a sameness about the timing of their action (insofar as it has been studied) suggesting that a single common process may be responsible for the termination of the susceptible stage, and for the irreversible stabilization of the genotype. The present paper is concerned primarily with the nature and determinants of the sensitive period.

Information about the labile phase of the post-irradiation period can be gathered from investigations of many different bacterial species, classes of mutations, and kinds of post-treatments. It has been found that usually the time during which the frequency of induced mutations is still subject to modification is limited to a fraction of the time required to complete the first post-irradiation cell division. To mention only some of the best-documented examples, this is true for photoreversibility of phage-resistance and other mutations in *Escherichia coli* (Kelner, 1949; Newcombe and Whitehead, 1951; Novick and Szilard, 1949), and of morphological mutations in *Streptomyces* (Newcombe, 1955); for temperature effects on phage-resistance (Witkin, 1953) and a variety of other mutations in *E. coli* (Berrie, 1953), and on mutations from auxotrophy to prototrophy in *Salmonella typhimurium* (Witkin, 1956); and for the effects of chloramphenicol and other conditions affecting the rate of protein synthesis on induced prototrophy in *E. coli* and *S. typhimurium* (Witkin, 1956), and on color variants on EMB agar in *E. coli* (Doudney and Haas, 1958). It appears to be generally true that the fate of the ultraviolet-induced "promutant" is irreversibly settled, one way or another, by the time the first half of the first post-irradiation cell division is accomplished.

It is known, too, that the process responsible for the termination of the sensitive period is dependent upon metabolic activity, for irradiated cells can maintain the capacity to react fully to post-treatments when incubated for relatively long periods in saline solution or buffer (Witkin, 1956; Doudney and Haas, 1958).

The temperature coefficient of the process responsible for the stabilization of the mutation frequency toward temperature changes (Witkin, 1953) and chloramphenicol (Doudney and Haas, 1958) has been found to be 2–3, and is identical with the Q_{10} of the rate of cell division in the same temperature range.

[1]These experiments were supported in part by grants from the National Science Foundation and the National Institute of Allergy and Infectious Diseases, and were conducted while the author was a Fellow of the Carnegie Institution of Washington.

[2]State University of New York, Downstate Medical Center, Brooklyn, N.Y., U.S.A.

The most provocative information about the sensitive period has come from studies in which the timing of cell division has been varied in more or less controlled ways. When the post-irradiation lag in cell division is prolonged by lowering the temperature, the sensitive period is lengthened proportionately, remaining a constant fraction (about one-third) of the lag (Witkin, 1953; Berrie, 1953). Newcombe (1955) has suggested that any agent capable of retarding cell division may extend the sensitive period, and he cites the effects of low temperature and of increasing doses of ultraviolet light on the period of photoreversibility of morphological mutants in *Streptomyces*. However, when the post-irradiation lag is lengthened by decreasing the concentration of amino acids in the medium, or by adding chloramphenicol (so that growth is limited by the rate of protein synthesis), the sensitive period remains almost constant in absolute time, and its duration is then quite independent of the length of the lag in cell division (Witkin, 1956). The stabilization of the mutation frequency toward post-treatments appears to be associated with the metabolism of cell division, but the association must be with an element of metabolism that is quite distinct from the synthesis of proteins.

These considerations have suggested the possibility that the fixation of the mutation frequency is accomplished through the synthesis of nucleic acids (perhaps through the duplication of genic DNA). If this is so, it is to be expected that the duration of the sensitive period should parallel the timing of the first post-irradiation cell division when the latter is prolonged by treatments known to inhibit nucleic acid synthesis.

In these experiments, the mutation followed was the change from auxotrophy to prototrophy, and the post-treatments used to study the sensitive period involved variations in conditions affecting the rate of protein synthesis in the period immediately after irradiation. It has been shown (Witkin, 1956) that the yield of induced prototrophs is directly proportional to the amount of protein synthesized during the sensitive period. Incubation of irradiated auxotrophs for 60 to 90 minutes in the presence of chloramphenicol, or in minimal medium without added amino acids, results in the stabilization of the mutation frequency at a low level, no longer susceptible to increase by restoration of active protein synthesis. Conversely, incubation under conditions permitting active protein synthesis for a comparable period of time guarantees a high yield of induced prototrophs which can no longer be reduced by subsequent chloramphenicol treatment.

Material and Methods

Bacterial strain. A tryptophane-requiring sub-strain of *Escherichia coli* B/r, designated as WP2, was used. Spontaneous mutations to prototrophy in this strain occur at a rate of about 10^{-8} mutations per bacterium per generation.

Preparation of cultures. Cultures of WP2 were grown in Difco nutrient broth, with the addition of 0.5 per cent NaCl, for 18 to 24 hours, at 37°C. with aeration. Cultures were centrifuged, and the pellets resuspended in sterile saline solution to give titers of about 1×10^9 cells per milliliter.

Irradiation. Saline suspensions were irradiated in 8-ml. lots in open petri dishes, with agitation during the exposure. The source was a General Electric germicidal lamp, having an intensity of 6.0 ergs per second per mm.^{2} at the distance used. At least 80 per cent of the output was of wavelength 2,537 Å. Yellow light was used to illuminate all operations during and after irradiation.

Post-treatments. Two methods of administering post-treatments were used. For

post-treatment on solid media, 0.2 ml. aliquots of irradiated suspension were spread on the surface of Difco nutrient agar, with the addition of 0.5 per cent NaCl, and with or without the addition of 20 units per milliliter of chloramphenicol. The plates were incubated for the desired times, after which the bacteria were washed off the surface in 3 ml. of sterile saline solution, with a glass spreader. The wash fluid was assayed for viable titer and prototroph frequency, either directly, or after centrifugation if necessary, to concentrate the cells. For post-treatment in tubes, the irradiated suspension was diluted 1:10 into the desired liquid, and assayed after incubation for suitable amounts of time. Post-treatments with chloramphenicol were always adminstered on plates, unless otherwise indicated since it was found that the amount of chloramphenicol carried over from treatment tubes onto final plating media was not negligible.

Assay methods and plating media. Assays for survival of the try$^-$ population and for induced prototrophs were made on the same medium, the "E" medium of Vogel and Bonner (consisting of inorganic salts with ammonium nitrogen), with 0.4 per cent glucose as a carbon source, and 5 per cent Difco nutrient broth (by fluid volume) added as partial enrichment. This semi-enriched medium contains enough trytophane to allow the formation of small, but distinctly visible, colonies by the parental auxotrophs when plated at high dilutions to determine survival. When dense populations are plated with little or no dilution, prototrophic mutants develop as large colonies, after two days of incubation, against a background film of limited parental growth. This medium, which will be referred to as SEM (semi-enriched medium), contains enough amino acids to guarantee a maximal rate of protein synthesis during the sensitive period (and hence a full yield of induced prototrophs) if the number of viable cells plated does not exceed $2–3 \times 10^7$.

Chloramphenicol was obtained through the courtesy of Charles Pfizer and Co. Caffeine and other inhibitory analogues used were obtained from the Nutritional Biochemicals Corporation.

Results

The Sensitive Period as a Function of Ultraviolet Dose

Apart from the use of thymineless mutants (an approach now under investigation), the use of ultraviolet light itself seemed the most effective way to change the timing of the first post-irradiation cell division by affecting the synthesis of nucleic acids (probably of DNA more or less specifically). Irradiation of *E. coli* B/r has been found to cause immediate inhibition of DNA synthesis, with relatively little effect on that of RNA or proteins at the low doses used in these experiments (Kelner, 1953; Kanazir and Errera, 1956). Since the ultraviolet-induced lag in cell division, which has been reported to be roughly proportional to dose, and the inhibition of DNA synthesis are both reversed by reactivating light (Kelner, 1954), the use of a series of increasing doses of ultraviolet light seemed a promising way to begin.

Figure 1 shows the growth curves obtained for WP2 after a series of ultraviolet treatments in the dose range from 0 to 540 ergs per mm.^{2}. As shown in Figure 2, the increase in lag is directly proportional to the dose in this range although there is little additional increase in lag as the dose is raised still higher (Beckhorn, 1952).

The Period of Sensitivity to Restoration of Protein Synthesis after Chloramphenicol Inhibition

In these experiments, the irradiated bacteria (after having received doses of 90, 180, 360, and 540 ergs per mm.^{2}) were spread on the surface of nutri-

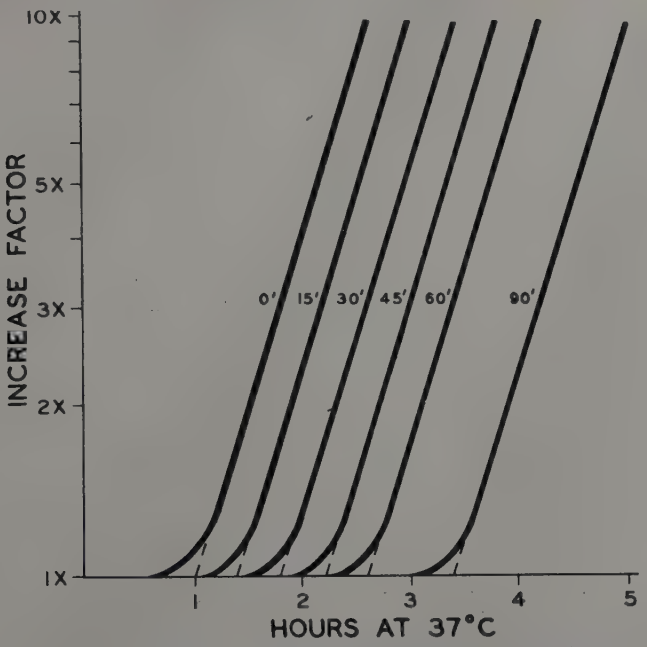


FIGURE 1. Growth of WP2 on nutrient agar. Bacteria irradiated in saline suspension (figure to left of each curve indicates dose in seconds); irradiated bacteria plated on nutrient agar (1.4×10^8 cells per plate at start), incubated at 37°C., washed from surface of several plates with 3 ml. saline solution at regular intervals, wash fluid assayed to determine viable titer.

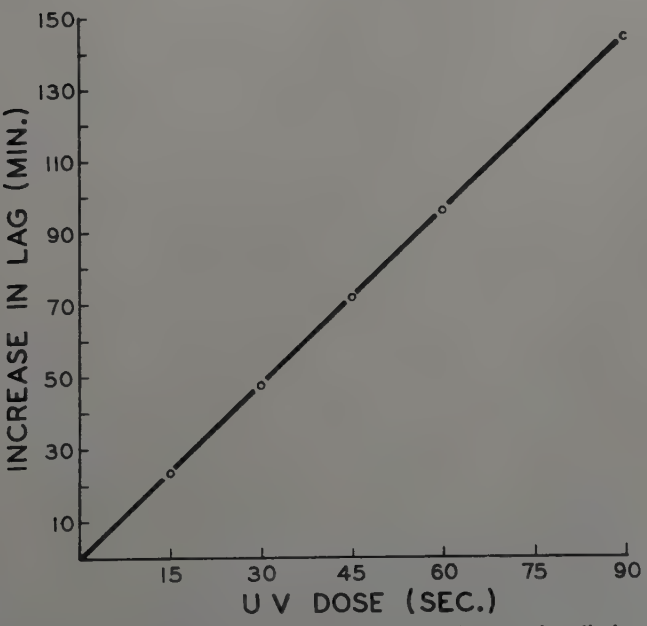


FIGURE 2. The increase in the length of the post-irradiation lag in cell division as a function of ultraviolet dose.

ent agar plates containing 20 units per milliliter of chloramphenicol, incubated for periods of time ranging from 0 to 90 minutes, then washed off the chloramphenicol agar and assayed to determine survival and mutation frequency. Replating on SEM medium constitutes restoration of conditions required for synthesis of proteins at the maximal rate, and failure to obtain the full yield of induced prototrophs on these plates is indicative of irreversible "loss" of mutants (whether through death or return to the genotype of the parental auxotroph cannot be determined). The sensitive period, therefore, is here defined as the time on chloramphenicol agar after which the restoration of protein synthesis no longer increases the mutation frequency above the base level obtained after prolonged treatment with chloramphenicol. Table I describes a typical experiment, and the results of a number of experiments are shown in Figure 3. The decline in mutant frequency with time on chloramphenicol is exponential until the base level is approached, and the sensitive period may, therefore, be given a numerical value by analogy with the "mean lethal dose" measure used to compare exponential survival curves. The time on chloramphenicol required to reduce the yield of mutants irreversibly to 37½

TABLE I

THE EFFECT OF INCREASED ULTRAVIOLET DOSE ON THE DURATION OF THE PERIOD OF SENSITIVITY TO RESTORATION OF PROTEIN SYNTHESIS*

Ultraviolet dose (sec.)	Time on chloramphenicol agar (min.)	Average no. of colonies on SEM plates: 10^{-5} dilution of wash (try$^-$ survivors)	Average no. of colonies on SEM plates: 10^{0} dilution of wash (induced try$^+$)	Mutations per 10^8 survivors	Percentage of maximum yield
15 (94% sur.)	0	395	54	137	100
	5	367	21	57	41.5
	10	408	11	27	19.7
	20	343	6	18	13.2
	40	389	6	14	10.2
	60	366	6	17	12.4
30 (71.3% sur.)	0	299	146	490	100
	10	316	72	228	46.5
	20	276	34	123	25.1
	40	304	21	70	14.2
	60	272	16	59	12.0
	90	248	15	60	12.2
60 (35.1% sur.)	0	147	392	2,670	100
	10	159	306	1,390	72.4
	20	154	176	1,140	42.6
	30	136	114	765	28.6
	40	121	72	595	23.1
	60	148	58	392	14.3
	90	119	30	252	9.4
90 (15.3% sur.)	0	64	394	6,150	100
	15	62	316	5,100	83.0
	30	46	142	3,200	52.0
	60	55	79	1,430	23.3
	90	44	42	955	15.5

*Irradiated WP2 spread on surface of nutrient agar containing 20 units per ml. of chloramphenicol; washed off at times indicated with 3 ml. of saline solution; wash fluid assayed for viable titer and prototroph frequency on SEM plates; incubations at 37° C.; data corrected for spontaneous mutations; final plating volume 0.1 ml.

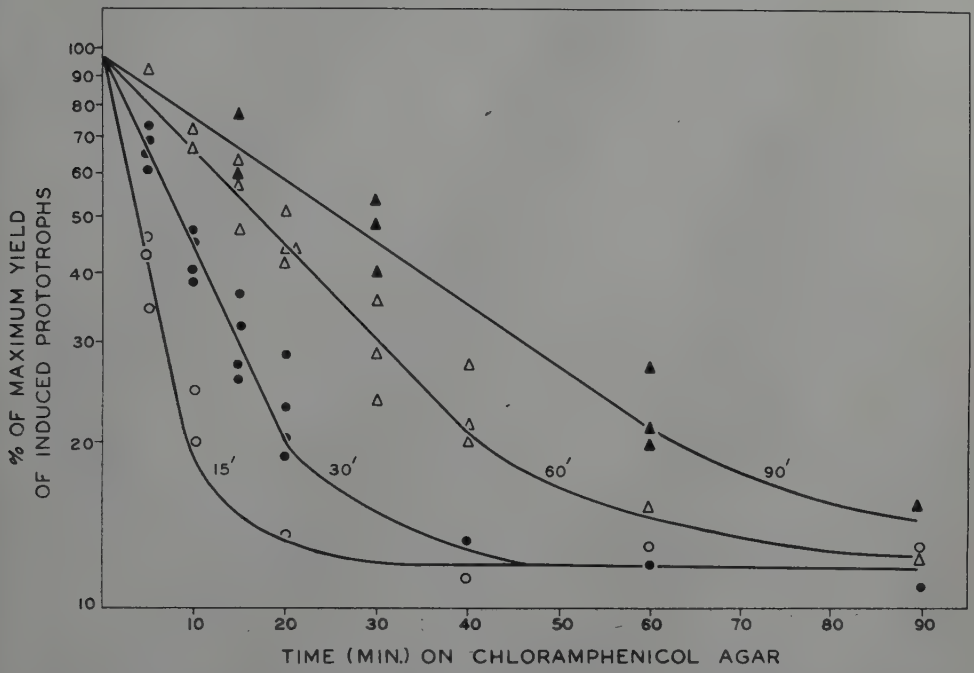


FIGURE 3. The effect of increased dose of ultraviolet light on the duration of the period of sensitivity to restoration of protein synthesis. See Table I for details of experiments; figure to right of each curve indicates ultraviolet dose.

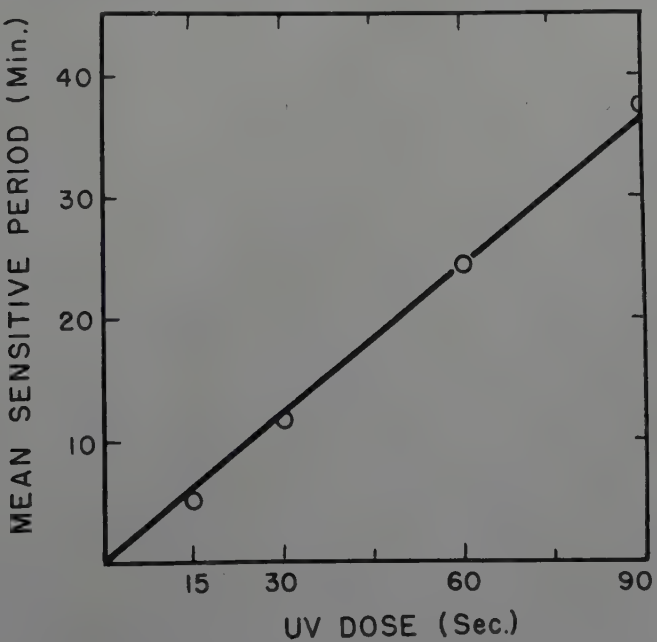


FIGURE 4. The effect of increased dose of ultraviolet light on the "mean sensitive period." The "mean sensitive period" is the time on chloramphenicol agar required to reduce the yield of induced prototrophs irreversibly to 37½ per cent of the maximum yield (see Fig. 3).

per cent of the maximum yield may be called the "mean sensitive period." Figure 4 shows that the increase in "mean sensitive period" is directly proportional to the dose of ultraviolet light.

The Period of Sensitivity to Inhibition of Protein Synthesis by Chloramphenicol

It has been shown that chloramphenicol or amino acid deprivation fails to reduce the yield of induced prototrophs if the irradiated bacteria are first incubated for 60 to 90 minutes under conditions permitting active protein synthesis. In this series of experiments, the sensitive period is defined as the time on nutrient agar (which supports active protein synthesis if the number of bacteria plated does not exceed 10^9) after which chloramphenicol challenge fails to reduce the yield of induced prototrophs below the yield normally obtained without any chloramphenicol treatment. Irradiated bacteria were spread on the surface of nutrient agar plates, incubated for periods of time ranging from 0 to 120 minutes, washed from the surface and concentrated by centrifugation. The concentrated suspension was assayed to determine survival and mutant frequency, and at the same time, aliquots were spread on the surface of nutrient agar plates containing chloramphenicol, incubated for one hour, and once again harvested and assayed for viable titer and number of prototrophs. A comparison of the mutation frequencies before and after the incubation with chloramphenicol tells the extent to which the mutation frequency has been irreversibly stabilized by the initial incubation on nutrient agar. Table II

TABLE II

THE EFFECT OF INCREASED ULTRAVIOLET DOSE ON THE DURATION OF THE PERIOD OF SENSITIVITY TO INHIBITION OF PROTEIN SYNTHESIS*

Ultraviolet dose (sec.)	Time on nutrient agar (min.)	Average no. of colonies per SEM plate: Wash I, 10^{-6} dil. (try^- survivors)	Wash I, 1:15 dil. (induced try)	Wash II, 10^{-5} dil. (try^- survivors)	Wash II, 10^{0} dil. (induced try^+)	Mutants per 10^8 survivors: Wash I	Mutants per 10^8 survivors: Wash II	Percentage of maximum yield
30	0	220	68	152	7	465	46	9.9
(66%	15	182	59	108	10	485	93	19.2
sur.)	30	242	72	158	28	446	177	39.6
	45	169	55	133	61	490	455	93.0
	60	202	66	146	78	485	534	100
60	0	112	158	76	16	2,100	210	10.0
(36.4%	20	99	191	63	23	2,900	366	12.6
sur.)	40	136	140	89	52	1,540	585	38.0
	60	111	166	72	123	2,250	1,710	76.0
	90	128	168	81	181	1,980	2,240	100
120	0	52	428	39	42	12,300	1,180	9.6
(3.9%	30	41	356	28	38	13,000	1,490	11.4
sur.)	45	38	326	22	53	12,900	2,410	18.6
	60	61	414	44	127	10,100	2,880	28.5
	90	55	391	39	228	9,550	5,850	61.0

*Irradiated WP2 spread on surface of nutrient agar (not more than 5×10^8 bacteria per plate); washed off at times indicated with 3 ml. of saline solution, wash fluid from 10 plates pooled, centrifuged, concentrated; concentrated wash (Wash I) assayed for viable titer and mutation frequency; aliquots of Wash I spread on surface of nutrient agar containing 20 units per ml. chloramphenicol, incubated 1 hr., washed with 3 ml .saline solution (Wash II); Wash II assayed for viable titer and frequency of prototrophs. Incubations at 37° C. Data corrected for spontaneous mutations. Final plating volume 0.1 ml.

gives a typical experimental protocol, and the data from several experiments are shown in Figure 5. If the stabilization of the mutation frequency at a high level (as in these experiments) or at low level (as in the preceding experiments) depends upon the same process, and if the rate of this process is the same whether or not protein synthesis is going on, one would expect these curves to be precisely recipro-

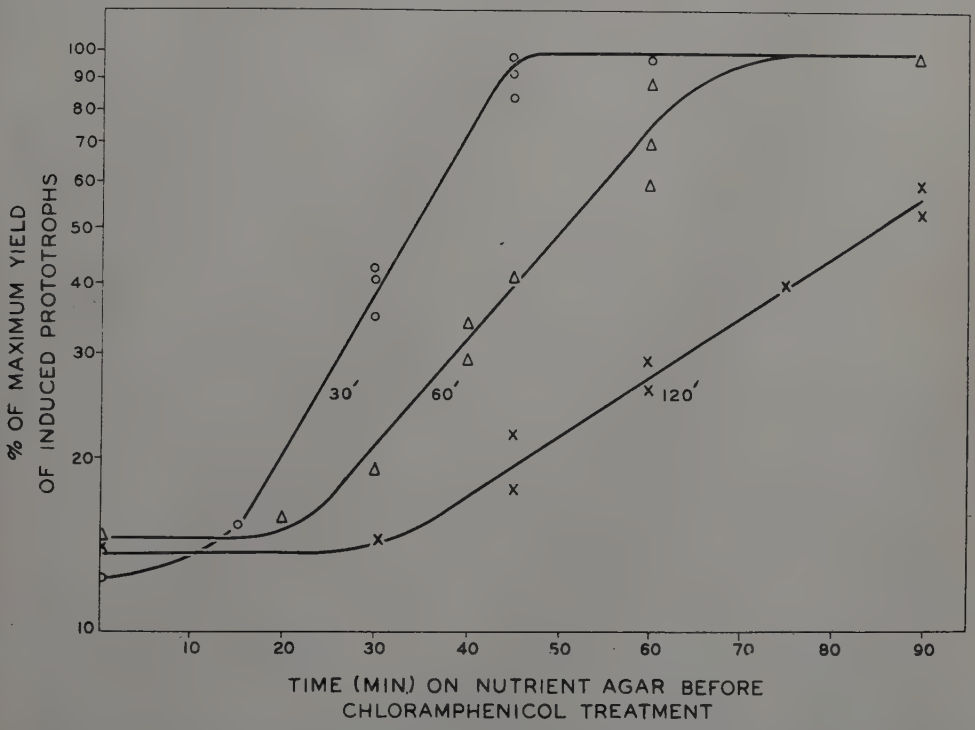


FIGURE 5. The effect of increased dose of ultraviolet light on the duration of the period of sensitivity to inhibition of protein synthesis. See Table II for details of experiments; figure to right of each curve indicates dose.

cal to those shown in Figure 3. The fact that they are not, differing most strikingly in the existence of a lag in the second set of curves, makes it seem likely that one or the other of these assumptions is not entirely correct. Of primary interest here, however, is the finding that the duration of the period during which chloramphenicol challenge can still reduce the yield of mutants is dose-dependent in the low-dose range. The time during which post-treatment can influence the mutation frequency grows longer as the dose of ultraviolet light is increased, whether conditions favor stabilization at a high level of mutation frequency or a low level. The duration of the sensitive period, therefore, parallels the timing of the first post-irradiation cell division when the latter is determined by ultraviolet dose.

The Effect of Caffeine and Other Purine and Pyrimidine Analogues on the Sensitive Period

The results just described relate the timing of the sensitive period to the particular metabolic activity that is made growth-limiting by irradiation with low doses of ultraviolet light, very likely the synthesis of DNA. Another way to change the

timing of cell division through effects on nucleic acid synthesis is the use of purine and pyrimidine analogues. A number of compounds in this category were screened for their effects on the post-irradiation lag in cell division and, although many were found to extend the lag, caffeine was selected as having a relatively wide range of concentrations in which the increased lag is proportional to the concentration,

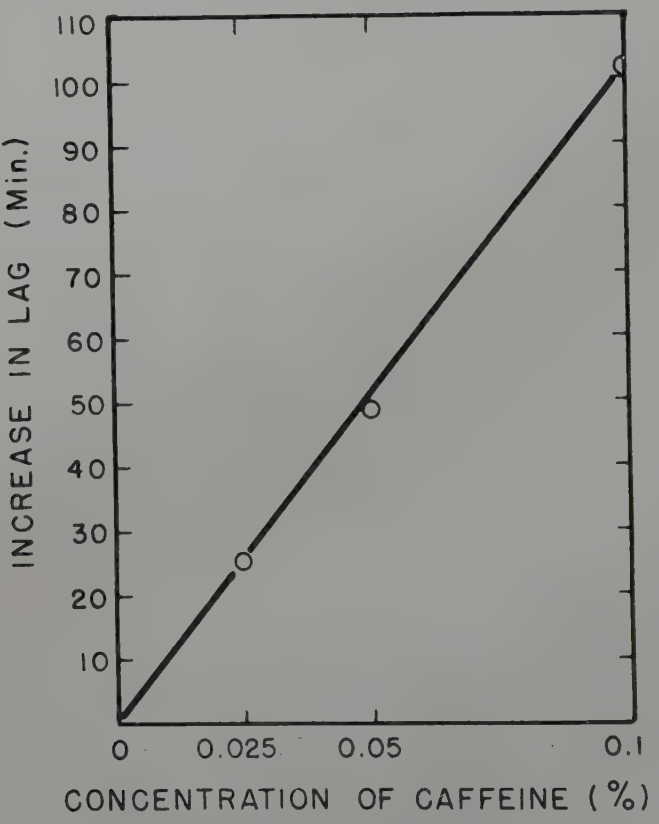


FIGURE 6. The increase in the length of the post-irradiation lag in cell division as a function of the concentration of caffeine in the growth medium. Lag defined as time required to accomplish doubling of inoculum of 1.1×10^7 survivors of irradiation with 90 ergs per mm.^{2} in 10 ml. of nutrient broth containing various concentrations of caffeine.

with no appreciable toxicity. Figure 6 shows the relation between caffeine concentration and increase in post-irradiation lag after a dose of 90 ergs. per mm.^{2}. Figure 7 shows results of experiments designed to determine the effect of caffeine on the sensitive period. Irradiated bacteria, in this series of experiments, were given post-treatment in tubes, and the sensitive period is defined here as the time in minimal medium (without exogenous amino acids) after which restoration of conditions required for active protein synthesis (plating out on SEM) can no longer raise the yield of prototrophs above the base level. Details of experimental procedure and results of a typical experiment are given in Table III.

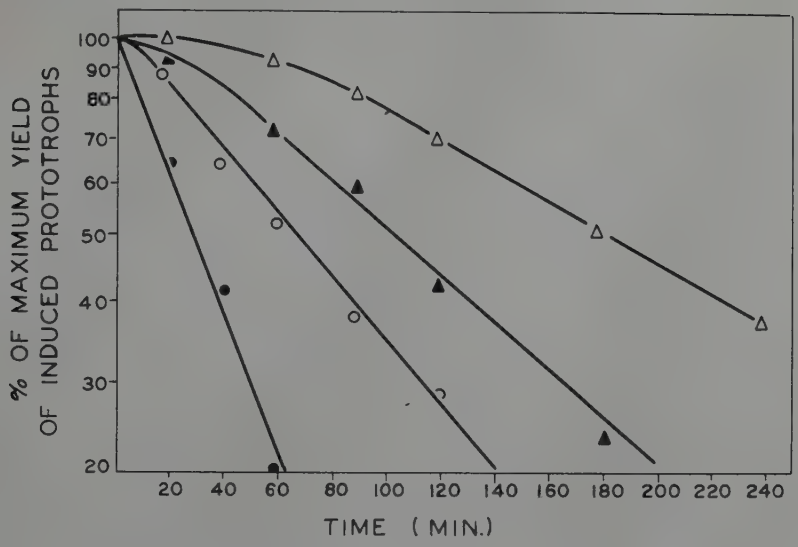


FIGURE 7. The effect of various concentrations of caffeine on the duration of the period of sensitivity to restoration of protein synthesis. Details of experiments given in Table III; closed circles, minimal medium without caffeine; open circles, minimal medium with 0.05 per cent caffeine; closed triangles, minimal medium with 0.1 per cent caffeine; open triangles, minimal medium with 0.2 per cent caffeine.

It is evident that the sensitive period is proportional to the concentration of caffeine, in the range of concentrations causing increasing extension of the post-irradiation lag in cell division.

Preliminary results obtained with another purine analogue, benzimideazole, are shown in Figure 8. When irradiated bacteria are incubated in minimal medium (or in nutrient broth plus chloramphenicol), the mutation frequency declines rapidly as measured by plating out on SEM plates, becoming stabilized at a level between 10 and 20 per cent of the maximum yield within one hour. Benzimideazole, when added to the minimal medium, brings about a decrease in the rate of decline, prolonging the sensitive period as did caffeine and increased ultraviolet dose. Similar results obtained in experiments with 2, 6 diamino purine sulphate and with 5-brom uracil suggest that many purine and pyrimidine analogues, used in concentrations that prolong the post-irradiation lag in cell division, may have similar effects.

The behavior of benzimideazole, as shown in Figure 8, is of particular interest since this compound can act in two different ways. As already indicated, it can cause a decreased rate of decline of the frequency of mutants in minimal medium. When benzimideazole is added to a medium containing a rich supply of amino acids, and in itself capable of supporting stabilization of the mutation frequency at the maximum level, the yield of prototrophs declines irreversibly with time, as it does under conditions of chloramphenicol inhibition or amino acid deprivation, except that the rate of decline is not so rapid. Thus, benzimideazole acts as if it affects the *rate* of stabilization through its effect on the rate of nucleic acid synthesis, as well as causing stabilization to occur at low level of mutant frequency, as if it is also able to inhibit the synthesis of proteins. This dual action would be expected, on the basis of the present interpretation, of an agent that is relatively non-specific in its inhibitory effects.

TABLE III

THE EFFECT OF INCREASING CONCENTRATIONS OF CAFFEINE ON THE DURATION OF THE PERIOD OF SENSITIVITY TO RESTORATION OF PROTEIN SYNTHESIS*

Concentration of caffeine	Time at 37° C. (min.)	Average no. colonies on SEM		Induced mutants per 10^8 survivors	Percentage of maximum yield induced mutants
		10^{-5} dil. (try^- survivors)	10^0 dil. (induced try^+)		
0	0	121	365	3,020	100
	20	144	240	1,680	55.4
	40	136	161	1,180	39.0
	60	141	86	610	20.2
	90	126	38	305	10.1
0.05%	0	135	344	2,550	100
	20	138	312	2,240	88.0
	40	118	218	2,020	79.0
	60	129	162	1,250	49.0
	90	144	122	850	33.3
	120	116	81	700	27.4
0.1%	0	111	388	3,480	100
	20	114	369	3,230	93.0
	60	123	274	2,220	64.0
	90	128	231	1,800	52.0
	120	141	213	1,510	43.5
	180	106	73	690	19.8
0.2%	0	133	356	2,680	100
	20	123	362	2,940	100
	60	122	303	2,480	92.5
	90	141	292	2,060	77.0
	120	126	253	2,000	74.6
	180	108	161	1,490	56.0
	240	117	124	1,060	39.8

*Irradiated WP2 diluted 1:10 into tubes containing minimal medium with concentrations of caffeine as indicated; tubes incubated and assayed at times shown to determine viable titer and prototroph frequency. Ultraviolet dose—60 sec. (survival 39.5%). Plating volume 0.1 ml. Data corrected for spontaneous mutations.

Some other facts of interest, shown also in Figure 8, may be worth pointing out here. It will be noted that incubation of irradiated cells in saline solution for 90 minutes results in only a very slight decline in the mutation frequency upon subsequent plating out on SEM medium. As would be expected, incubation in nutrient broth with a supplement of casein hydrolysate for 90 minutes guarantees the full yield of prototrophs upon plating out. (In incubation in nutrient broth, however, the mutation frequency is by this time irreversibly fixed at the high level, whereas incubation in saline merely postpones stabilization, which may occur at a high level, if plated out directly on SEM, or at a low level, if an exposure to chloramphenicol precedes the plating out on SEM.) This figure also shows that the decline of the mutation frequency occurs much more slowly in minimal medium lacking a carbon source than in the presence of glucose, indicating an energy requirement for stabilization. Finally, it is also shown that the rate of decline of the mutation frequency is about the same whether the method used to inhibit protein synthesis is incubation with chloramphenicol (in the presence of amino acids), or incubation in minimal medium without amino acids.

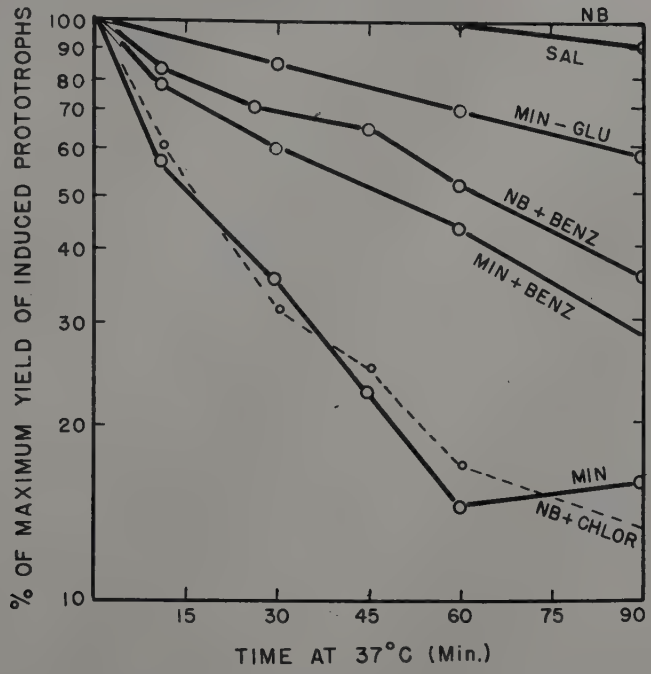


FIGURE 8. Effects of various post-treatments on the sensitive period. Irradiated WP2 (60-sec. dose) diluted 1:10 into tubes containing the following liquids: min.—minimal medium; n.b. + chlor.—nutrient broth (with 2 mg. per ml. casein hydrolysate added) plus 20 units per ml. chloramphenicol; min. + benz.—minimal medium plus 0.2 per cent benzimideazole; n.b. + benz.—nutrient broth (with 2 mg. per ml. casein hydrolysate added) plus 0.2 per cent benzimideazole; min.-gl.—minimal medium without glucose; sal.—saline solution; n.b.—nutrient broth (with 2 mg. per ml. casein hydrolysate added).

A Reinterpretation of Non-linear Dose-mutation Curves

The results described thus far have immediate implications for the interpretation of the puzzling relationship that has often been found between ultraviolet dose and the frequency of induced bacterial mutations. The steep rise of mutation frequency with dose that is found for induced prototrophs in strain WP2 (lower curve in Figure 9) is typical of the departure from the expected linear function that is characteristically obtained in studies involving ultraviolet-induced prototrophy. A recent study has contrasted this type of curve with the linear curves obtained in other mutational systems in bacteria (Zelle, Ogg, and Hollaender, 1958).

A possible explanation for the steep rise of mutation frequency with dose follows directly from consideration of two of the results obtained in these investigations: (i) the yield of induced prototrophs is proportional to the amount of protein synthesized during the sensitive period, and (ii) the duration of the sensitive period is proportional to the dose of ultraviolet light in the low-dose range. Thus, a doubling of the dose, in this range, not only causes a doubling of the number of "promutants" by doubling the number of "hits" (the expected linear increase), but it also doubles the length of time during which the synthesis of proteins (very little

affected by low doses of ultraviolet light) can still influence the yield of actual prototrophs obtained. According to this analysis, there is a large population of potential mutants induced at low doses that never become prototrophs because the time before the stabilization of the mutation frequency is completed is too short to

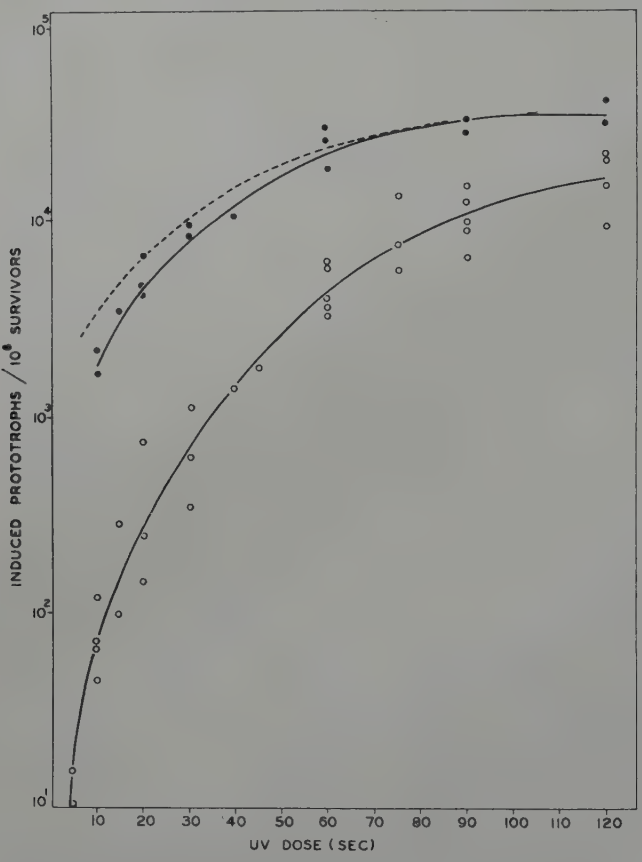


FIGURE 9. The effect of caffeine on the relation between ultraviolet dose and frequency of induced prototrophs. See Table IV for details of experiments; open circles, SEM plates used to determine survival and mutation frequency; closed circles, SEM plates containing 0.1 per cent caffeine used to determine survival and mutation frequency. Survival curves shown in Figure 10. Broken line indicates theoretical linear function extrapolating backward from highest mutation frequency obtained.

permit much protein synthesis. As the dose increases, the fraction of promutants actually becoming prototrophs also increases, as a result of the longer time available, before the sensitive period, during which the synthesis of proteins can still influence the yield, is terminated.

The results with caffeine suggested a way to test this interpretation. In these experiments, caffeine itself exerts no mutagenic effect. It causes prolongation of both the sensitive period and the lag in cell division that is proportional to the concentration of caffeine over a considerable range. It seemed possible, therefore, that

if bacteria irradiated with low doses of ultraviolet light were plated on SEM containing the highest possible non-toxic concentration of caffeine, the sensitive period might be extended far beyond its usual duration after low-dose irradiation, and the hypothetical crop of potential mutants might then have sufficient time during which the synthesis of proteins could influence their fixation as prototrophs. It would be expected, according to this reasoning, that a mutation-dose curve obtained by plating on SEM containing caffeine would approach linearity, and would cause enhancement of the mutagenic effects of ultraviolet that would be relatively greater at the lowest doses than at high doses. Figure 9 shows the results of experiments designed to compare the mutation-dose curves for induced prototrophs when the

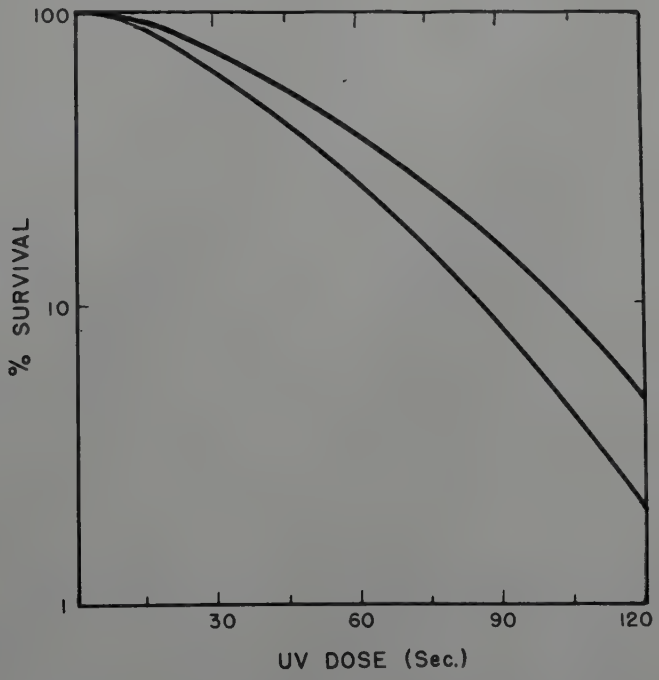


FIGURE 10. Survival of WP2 after various doses of ultraviolet light. See Table IV for details of experiments; upper curve, irradiated bacteria plated on SEM plates; lower curve, platings on SEM containing 0.1 per cent caffeine.

irradiated bacteria are plated on SEM agar with those obtained when the determinations of survival and mutation frequency are made on SEM agar containing 0.1 per cent caffeine. Survival curves on the two kinds of plates are shown in Figure 10. As may be seen in the sample experiment shown in Table IV, caffeine alone shows no mutagenic activity whatsoever in this material. Nevertheless, the addition of this material to the post-irradiation medium causes a dramatic enhancement of the mutagenic effect of ultraviolet light, with the greatest relative effect at the lowest doses, as predicted. The results are consistent with the hypothesis that the enhancement of the mutagenic effect of ultraviolet light by caffeine is a consequence of the prolongation of the sensitive period.

TABLE IV

THE EFFECT OF CAFFEINE ON THE RELATION BETWEEN ULTRAVIOLET DOSE AND THE FREQUENCY OF INDUCED PROTOTROPHS*

	Try⁻ survivors				Induced prototrophs					
	SEM plates		SEM-caffeine plates		SEM plates			SEM-caffeine plates		
Ultraviolet dose (sec.)	Dilution	Average no. colonies	Percentage survival	Average no. colonies	Percentage survival	Dilution	Average no. colonies	Mutations per 10^8 survivors	Dilution	Average no. colonies	Mutations per 10^8 survivors
0	10^{-6} (0.1)	185	100	193	100	10^{-1} (0.1)	8	—	10^{-1} (0.1)	5	—
10	10^{-6} (0.1)	179	97.0	178	93	10^{-1} (0.2)	27	75	10^{-1} (0.1)	246	1,380
20	10^{-6} (0.1)	154	83.5	142	74	10^{-1} (0.2)	97	310	10^{-2} (0.1)	73	5,140
30	10^{-6} (0.1)	133	72.0	114	59	10^{-1} (0.1)	121	910	10^{-2} (0.1)	86	7,550
40	10^{-6} (0.1)	109	59.0	89	46	10^{-1} (0.1)	141	1,290	10^{-2} (0.1)	88	9,900
60	10^{-6} (0.2)	142	38.4	80	20.8	10^{-2} (0.2)	46	3,260	10^{-2} (0.1)	82	20,200
90	10^{-5} (0.1)	273	14.8	139	7.2	10^{-2} (0.1)	42	7,700	10^{-2} (0.2)	73	26,300
120	10^{-5} (0.2)	172	4.6	46	1.2	10^{-1} (0.1)	80	9,350	10^{-1} (0.1)	87	38,000

*WP2 irradiated additively; after every dose increment an aliquot of the irradiated suspension was removed and assayed for survival and prototroph frequency on SEM plates with and without caffeine (0.1%). Figure in parentheses under dilution indicates volume plated. Data corrected for spontaneous mutations.

DISCUSSION

These experiments leave little room for doubt that the stabilization of the mutation frequency toward post-treatments is intimately associated with the metabolism of cell division. The direct proportionality of the sensitive period to the lag in cell division when the latter is prolonged by increasing doses of ultraviolet light, or by increasing concentrations of caffeine, suggests that the stabilization is accomplished through the particular pathway that is most persistently inhibited by each of these agents, and that thereby becomes the limiting factor in determining the timing of the first cell division. Protein synthesis can be dismissed readily as the determinant of the timing of the sensitive period, since stabilization of the mutation frequency occurs at a rate that is independent of the lag in cell division when protein synthesis is differentially inhibited. Furthermore, there is ample evidence that the inhibition of cell division caused both by ultraviolet light (in the low-dose range) and by caffeine is primarily the consequence of effects on the synthesis of nucleic acids. The bulk of the evidence available for ultraviolet light implicates DNA as the primary site of inhibition, although a recent study reports inhibition of RNA synthesis as well in populations past the logarithmic phase of growth (Iverson and Giese, 1957).

The inhibition of cell division by caffeine is partially reversible by guanine and other purines (Fries and Kihlman, 1948) and is thought to be caused primarily by

competition for purine-specific enzymes. The well-known genetic effects of caffeine, including its ability to break chromosomes and to induce mutations (Kihlman and Levan, 1951; Novick, 1955), are also reversible by purines and by nucleosides (Novick, 1955; Gersh, unpublished), and constitute presumptive evidence that its possible sites of action must include genic DNA. The parallel effects of caffeine and ultraviolet light on the extension of the lag in cell division and on the duration of the sensitive period are best explained by assuming overlapping mechanisms of inhibition, with a common limiting factor determining the timing of recovery from inhibition. Whatever this limiting step may prove to be (and it is almost certain to involve the synthesis of DNA or RNA, or both), it is intimately related to the stabilization of the mutation frequency. It seems quite certain that induced mutations become impervious to the modifying influence of post-treatments as the results of a process associated with the synthesis of nucleic acids. Whether or not the mutation frequency is stabilized at a high level or at a low level depends (in the case of induced prototrophs) upon the amount of protein synthesis that takes place before some critical phase of nucleic acid elaboration (gene duplication?) is accomplished.

Doudney and Haas (1958), who have found that the frequency of color variants on EMB agar after irradiation of *E. coli* B. responds to post-treatments in much the same way as do most induced prototrophs, have expressed the opinion that "mutation frequency decline," the drop in mutation yield that occurs when the irradiated cells are incubated with chloramphenicol, or without amino acids, is not associated with any of the major macromolecular syntheses. This conclusion is rather difficult to reconcile with the way in which the rate of "mutation frequency decline" varies with the dose of ultraviolet light, and with the concentration of caffeine, in just the ranges of dose or concentration also causing proportional extension of the lag in cell division. Doudney and Haas base their opinion primarily upon the speed of the process of "mutation frequency decline" which, in their material, is essentially completed in fifteen minutes, too soon, they believe, to reflect a significant amount of synthetic activity. Actually, the rapidity of stabilization of the mutation frequency in the Doudney and Haas experiments can be used as an argument in favor of its association with nucleic acid synthesis. According to the procedure they outline, irradiation of the bacteria was preceded by a fifty-minute incubation in minimal medium, which means that the population was in the late stages of the lag phase when the ultraviolet treatment was given. Experiments designed to compare the timing of the sensitive period when bacteria at different stages of division are irradiated show that late-lag phase cells are characterized, not only by a much shorter sensitive period than is found for "stationary" phase bacteria, but also by a much shorter post-irradiation lag. The relatively greater dependence of "mutation frequency decline" upon a source of carbon found in these experiments, as compared with those of Doudney and Haas, seems also to be a consequence of the difference in the culture phase.

The assumption that a single process determines the period of susceptibility to all post-irradiation influences, however varied their points of attack and routes of action, is strengthened by the results obtained when different doses of ultraviolet light are used. Whether the conditions of post-treatment favor low yields of induced prototrophs (chloramphenicol) or high yields (nutrient agar), the time during which a change in the rate of protein synthesis can still influence the mutation frequency is similarly affected by increasing the dose. Proportionality of the sensitive period to ultraviolet dose in the low-dose range is apparently not confined

to post-treatments directly involving protein synthesis. Newcombe (1955) found that the timing of photoreversibility of morphological mutants in *Streptomyces* responded to dose in this way, and Matney *et al.* (1958), working with very low doses (around 4 ergs per mm.^{2}) that do not kill, have reported an extremely short sensitive period for photoreversibility of induced streptomycin resistance.

A comparison of the kinetics of mutation frequency stabilization under conditions favoring low and high yields (and assuming that the same process is being measured) reveals important differences. In the presence of chloramphenicol, or in unsupplemented minimal medium, stabilization begins immediately after irradiation, and proceeds exponentially until the base level is approached, at a rate that depends upon the dose of ultraviolet light. When amino acids are abundantly supplied, and chloramphenicol is absent, stabilization begins only after a lag, and then proceeds exponentially at a rate that is slightly faster (at a given dose) than the rate of decline in chloramphenicol, and that is also dose-dependent. If the assumption is made that the stabilization of the mutation frequency, in either direction, reflects the rate of a step in gene duplication, it follows that the onset of the latter is delayed, and its rate slightly increased, under conditions permitting concurrent protein synthesis. This difference in timing suggests that the mechanism of gene duplication may actually be quite different when protein synthesis is going on than when it is differentially inhibited, and herein may lie the key to the role of protein synthesis in mutagenesis. These speculations, however, are something of a digression, since it is the limited goal of the present paper to discuss the question of timing, rather than to attempt to reconstruct the pathway through which any particular post-treatment exerts its influence.

The preliminary results obtained with purine and pyrimidine analogues add to the evidence that mutation frequency stabilization is related to the timing of nucleic acid synthesis, since the concentrations that were effective in extending the sensitive period invariably caused prolongation of the post-irradiation lag in cell division, as well. The dual action of benzimideazole, which has already been mentioned, is of special interest. This compound, which is considered to be an analogue of guanine (Wooley, 1952), decreases the rate of decline in mutation frequency that occurs in unsupplemented minimal medium in much the same way as do caffeine and increased doses of ultraviolet light. When amino acids are present, however, benzimideazole has a double effect. It causes a decline in mutation frequency, as if it were decreasing the rate of protein synthesis differentially, but at the same time it extends the period during which the decline takes place, as if it is also affecting the rate of the process responsible for mutation-frequency stabilization. The action of benzimideazole essentially duplicates the effects described by Doudney and Haas (1958) for 5-hydroxyuridine, and a similar pattern of effects might be expected from any inhibitor that acts rather non-specifically, affecting the synthesis of proteins and nucleic acids. Inhibition of protein synthesis has been described for benzimideazole (Creaser, 1955).

The effect that caffeine was found to have on the mutation-dose curve, precisely that predicted on the basis of its effect on the sensitive period, should, theoretically, be duplicated by other inhibitors that prolong cell division by acting, either specifically or differentially, upon the particular synthetic pathway related to mutation-frequency stabilization, with relatively little effect on protein synthesis. Pretreatment with nitrogen mustard has been found to cause considerable enhancement of induced mutation frequencies in *Aspergillis* and *Neurospora* (Swanson and Goodgal, 1947), at concentrations which, alone, are only slightly mutagenic.

According to the interpretation offered here, the exponential rise of induced prototroph frequency with ultraviolet dose in the low-dose range is a consequence of the added time that each dose increment provides during which the mutation yield can be influenced by the continued synthesis of proteins. Any mutational system similarly dependent upon protein synthesis after irradiation should exhibit the same type of non-linear frequency-dose relation, and should also respond to post-treatment with caffeine, with the greatest relative enhancement at the lowest doses. It has been found, in the course of studies of the generality of the requirement for post-irradiation protein synthesis in mutagenesis, that these factors (non-linear mutation-dose curve, enhancement by caffeine) do form a constellation with susceptibility to the antimutagenic effect of chloramphenicol post-treatment. For example, mutations from streptomycin dependence to independence exhibit a linear relation between ultraviolet dose and induced mutation frequency (Zelle, Ogg, and Hollaender, 1958), are not affected by post-treatment with chloramphenicol, or by other treatments that inhibit protein synthesis differentially, and fail to show the slightest enhancement by caffeine. Ultraviolet-induced mutations from lactose non-utilization to utilization, on the other hand, display non-linear dose-mutation curves and are suppressed by chloramphenicol post-treatment and enhanced by caffeine, as are most mutations from auxotrophy to prototrophy.

This report has been concerned with the timing of post-irradiation influences on mutations, and in it I have attempted to discuss not *how* protein synthesis affects induced mutations, but, less ambitiously, *when* it does so. Definitive answers to both of these questions, as to many others relating to genes and their ways of changing, will surely be among the fruits of the current effort to understand the molecular basis of genetic specificity and the mechanisms by which it is maintained and transferred.

Summary

The experiments described in this report were designed to obtain information about the timing of susceptibility to post-irradiation influences on the frequency of induced mutations. Mutations to prototrophy, induced by ultraviolet light in a tryptophane-requiring sub-strain of *E. coli* B/r, were investigated. These mutations belong to a class that responds to post-treatments affecting protein synthesis during a "sensitive period" immediately after irradiation. The following new facts were established:

(i) When the timing of the first post-irradiation cell division is altered by varying the dose of ultraviolet light, the timing of the sensitive period is correspondingly changed; in the range of doses below 1,000 ergs per mm.^{2}, the retardation of cell division and the duration of the sensitive period (during which the yield of prototrophs could still be decreased by chloramphenicol, or increased by removal of chloramphenicol) were both found to be proportional to dose.

(ii) When the first post-irradiation cell division is retarded by adding caffeine to the medium, the sensitive period is prolonged. In the range of concentrations between 0.05 per cent and 0.2 per cent, the extension of the lag in cell division and the prolongation of the sensitive period are both proportional to concentration.

(iii) Concentrations of benzimideazole, 2,6 diamino purine and 5-brom uracil that retard cell division were also found to prolong the time during which the frequency of ultraviolet-induced prototrophs remained susceptible to post-irradiation influences. These results, considered in the context of facts already known, have led to the conclusion that the process leading to the termination of the sensitive period,

and to the irreversible fixation of the mutation frequency, is intimately associated with the synthesis of nucleic acids during the first post-irradiation cell division.

An interpretation is offered to account for the non-linear relation between mutation frequency and ultraviolet dose that is often observed in bacteria. It is proposed that an increase in dose, in the low-dose range, not only causes an increase in the number of "promutants," but also, by extending the sensitive period, allows more time for the continued synthesis of proteins to influence the establishment of a larger fraction of potential mutants as actual prototrophs. It is predicted that caffeine, which extends the sensitive period without itself inducing mutations (in this material), should duplicate the secondary effects of increased ultraviolet dose, causing the dose-mutation curve to approach linearity. Caffeine was found to have the anticipated effect, dramatically enhancing the mutagenic effect of low doses of ultraviolet light.

Acknowledgments

The author is deeply grateful to Mrs. Mary Ruth Hampton and to Mrs. Elizabeth C. Theil for their efficient and enthusiastic assistance in carrying out these experiments.

References

Beckhorn, E. J. 1952. Photoreversal of the lengthening effect of ultraviolet radiations on the bacterial lag period. J. Bact., Balt. *63*: 687–688.

Berrie, A. M. M. 1953. The effects of temperature on ultraviolet-induced mutability in *Escherichia coli*. Proc. Nat. Acad. Sc. U.S. *39*: 1125–1133.

Creaser, E. H. 1955. Inhibition of induced enzyme formation by purine analogues. Nature *175*: 899–900.

Demerec, M., G. Bertani, and J. Flint. 1951. A survey of chemicals for mutagenic action on *E. coli*. Am. Natur. *85*: 119–136.

Doudney, C. O., and F. L. Haas. 1958. Modification of ultraviolet-induced mutation frequency and survival in bacteria by post-irradiation treatment. Proc. Nat. Acad. Sc. U.S. *44*: 390–401.

Fries, N., and B. Kihlman. 1948. Fungal mutations obtained with methyl xanthines. Nature, Lond. *162*: 573–574.

Gersh, E. S. Unpublished, quoted in Novick (1955).

Iverson, R. M., and A. C. Giese. 1957. Synthesis of nucleic acids in ultraviolet-treated *Escherichia coli*. Biochim. biophys. acta, Amst. *25*: 62–68.

Kanazir, D., and M. Errera. 1956. Alterations of intracellular deoxyribonucleic acid and their biological consequence. Sympos. Quant. Biol. *21*: 19–29.

Kelner, A. 1949. Photoreactivation of ultraviolet-irradiated *Escherichia coli*, with special reference to the dose-reduction principle and to ultraviolet-induced mutation. J. Bact., Balt. *58*: 511–522.

——— 1953. Growth, respiration and nucleic acid synthesis in ultraviolet-irradiated and in photoreactivated *Escherichia coli*. J. Bact., Balt. *65*: 252–262.

——— 1954. The specific inhibition of DNA syntheses by ultraviolet light and its reversal by reactivating light, pp. 121–125. Proc. 1st Internat. Photobiol. Congr. Wageningen: H. Veenman and Zonen.

Kidder, G. W., and V. C. Dewey. 1949. The biological activity of substituted purines. J. Biol. Chem. *179*: 181–187.

Kihlman, B., and A. Levan. 1951. Localized chromosome breakage in *Vicia faba*. Hereditas, Lund. *37*: 382–388.

Matney, T. S., D. M. Shankel, and O. Wyss. 1958. Mutations induced by ultraviolet light without attendant lethality. J. Bact., Balt. *75*: 180–183.

NEWCOMBE, H. B. 1955. The timing of induced mutations in *Streptomyces*. Brookhaven Sympos. Biol. *8*: 88–102.

NEWCOMBE, H. B., and H. A. WHITEHEAD. 1951. Photoreversal of ultraviolet-induced mutagenic and lethal effects in *Escherichia coli*. J. Bact., Balt. *61*: 243–251.

NOVICK, A. 1955. Mutagens and antimutagens. Brookhaven Sympos. Biol. *8*: 201–215.

NOVICK, A., and L. SZILARD. 1949. Experiments on light-reactivation of ultraviolet inactivated bacteria. Proc. Nat. Acad. Sc. U.S. *35*: 591–600.

SWANSON, C. P., and S. H. GOODGAL. 1947. The effect of nitrogen mustard on the ultraviolet-induced mutation rate in *Aspergillis terreus*. Rec. Genet. Soc. Am. *16*: 54; Genetics *33*: 127 (1948).

WITKIN, E. M. 1953. Effects of temperature on spontaneous and induced mutations in *Escherichia coli*. Proc. Nat. Acad. Sc. U.S. *39*: 427–433.

——— 1956. Time, temperature, and protein synthesis: A study of ultraviolet-induced mutation in bacteria. Sympos. Quant. Biol. *21*: 123–140.

WOOLEY, D. W. 1952. A Study of Antimetabolites. New York: Wiley & Sons. Pp. 269.

ZELLE, M. R., J. E. OGG, and A. HOLLAENDER. 1958. Photoreactivation of induced mutation and inactivation of *Escherichia coli* exposed to various wave lengths of monochromatic ultraviolet radiation. J. Bact., Balt. *75*: 190–198.

METABOLISM AND THE STABILITY OF CHROMOSOMES[1]

C. P. Swanson, T. Merz, and N. Cohn[2]

THE TITLE of this paper is both vague and presumptuous: vague because it gives no certain clue as to the direction and purpose of the discussion, presumptuous because of the meagerness and somewhat tenuous nature of the data upon which this discussion must rest. Looked at from the present trend of research, however, the title is both appropriate and timely, and it is this which gives us courage to attempt a reassessment of our knowledge in the field and to redirect our thinking, if only for the moment.

It must be patently obvious to students of the chromosome that the nucleus both controls and is, in turn, controlled by the metabolism of the cell as a whole. The constancy of genes and chromosomes through innumerable cell divisions speaks of a profound stability under a wide variety of cellular circumstances, a situation all the more remarkable since this stability is dynamic rather than static. Being dynamic, structural and functional integrity is maintained only at the expense of energy derived from metabolic processes, either cytoplasmic or nuclear, or both. Loss or diversion of this energy should, therefore, be reflected in nuclear alterations of varying degrees of severity and permanence.

In our studies we have used the frequency of chromosomal aberrations as a measure of chromatin stability. Although gross in nature, and far removed from initial events, aberrations have the advantage of being easily and accurately scored, and of being quantitatively reproducible. An extensive literature indicates that the sensitivity of chromosomes to induced breakage and rejoining can be varied so that the final frequency of aberrations resulting from any given exposure may be amplified or diminished rather profoundly. When such variations in frequency are possible, as with X-rays and certain chemical mutagens, it appears that there exists, between the introduction of radiant energy or a chemical into the cell and the realization of a completed aberration, a chain of events which involves, in a manner not yet fully understood, the metabolic machinery of the cell. Oxygen is intimately involved in the reactions which take place. With X-rays, oxygen is instrumental in modifying, probably through the medium of active radicals, the effective geometry of ion paths and, in a less direct manner, the recovery processes of the cell. Some effective chemicals, but not all, are also modified in their action by an alteration in oxidative metabolism, that is, respiration and phosphorylation. The metabolic processes are thereby implicated in the expression of chromosomal damage, but what is not quite so evident is the question of whether the state of the chromosome, structurally and functionally, is unvarying under these diverse conditions of exposure. If the chromosome is altered by a changed environment, which seems more than likely, one must search for those factors which alter the state of the chromosome and which, at the same time, affect its stability as reflected in its spontaneous as well as its induced rate of change.

[1]That part of the authors' work reported herein was supported in part by the U.S. Atomic Energy Commission, the National Institutes of Health, and the National Science Foundation.

[2]Department of Biology, The Johns Hopkins University, Baltimore 18, Md., U.S.A.

Let us consider the chromosomes in the living interphase nucleus, the stage of the division cycle when most exposures are made and in which most chromosomal effects later observed are produced. The chromosomes are in a swollen, extended state, and usually fill the nuclear area so completely that they obliterate any semblance of linear structure except for the nucleoli and regions of heterochromatin. Although the events which transform the compacted chromosome of telophase into the extended one of interphase are imperfectly understood, no one questions the fact that the chromosome is a highly complex nucleoprotein gel, a macromolecular system whose unique qualities depend upon a labile morphological foundation adapted for highly specific syntheses (replication) and informational transfers, on the one hand, and for mitotic and meiotic events, on the other. Water, of course, is an integral part of the gel, being squeezed out as the chromosomes contract and progress toward metaphase, and taken up in the reorganization and expansion of the telophase nucleus. An abundance of water is associated with the actively metabolic nucleus, a lack with its relative inertness during division. We may legitimately ask, therefore, if these changes relate in any way to chromosome stability. An affirmative answer seems obvious even though the supporting evidence is meager and often conflicting.

As a complex hydrophilic fabric, chromatin can have its physical state modified by environmental changes much in the manner of simple gels. As Ris and Mirsky (1949) have demonstrated, neutral salts, changes in temperature and pH, and anoxia markedly alter the appearance of the interphase nucleus, changing it in such a way that its usual optical homogeneity is lost and a pronounced heterogeneity of structure results. The alterations are readily reversible, but what is of immediate importance is that these environmental variables are the ones customarily employed in studying the effectiveness of different mutagens under a variety of exposure conditions, and which implicate cellular metabolism in determining the final frequency of aberrations. It is this avenue of approach to an understanding of chromatin stability which will be explored.

It has long been known that different organisms, cells, chromosomes, and genes show varying degrees of sensitivity to mutagenic agents, and exhibit varying degrees of spontaneous instability. Sparrow (1951) has extensively reviewed this subject, particularly as it relates to the stability of the chromosomes. Except to point out that a genetic factor for stability is probably operative, he finds no evident common denominator which permits one as yet to relate stability to any physical or chemical state of the cell as a whole, or of any part thereof. However, irreparable damage to the cell is generally of nuclear origin, so that it is laboring the obvious to state that the problem is really a nuclear one involving colloid chemistry of an intricate and delicately balanced sort. Sparrow, for example, has beautifully demonstrated, and others have since corroborated his findings, that there is a progressive increase in the breakage of chromosomes by X-rays as cell division proceeds, with metaphase chromosomes being the most fragile. The sequence of sensitivity follows, in general, the dehydration of the chromatin gel as the chromosomes undergo their contraction in preparation for anaphase separation. Since the essential syntheses necessary for chromatin replication have already occurred, the progressive elimination of water from the gel and the accompanying molecular reorganization appear in this instance to be responsible for the progressive increase in sensitivity. Although difficult to demonstrate directly, metabolic energy is undoubtedly necessary not only for gel transformation but for its integrity as well. But the picture is by no means clear-cut. Ehrenberg (1955), for example, has found that an increased

water content of barley seeds (up to 18 per cent) leads to an increase rather than a decrease in the amount of radiation-induced damage with X-rays, although not with neutrons. There is no certainty that Ehrenberg's data are in conflict with the general concept advanced here that dehydrated chromatin is more unstable to radiation than is hydrated chromatin. The contradiction may be more apparent than real since an increased water content leads to a more metabolically active seed, and metabolism, as we shall attempt to show, plays a decided role in the determination of chromosomal damage.

In a more direct sense, Wolff and Luippold (1955) and Steffensen (1957) have demonstrated the role of metabolism in controlling aberration rates. Through fractionation studies with X-rays, the former investigators have found that the rejoining of broken ends of chromosomes is an energy-requiring event involving oxidative phosphorylation, with the energy necessary for rejoining very probably derived from high energy phosphate bonds. Nuclear RNA could also serve as a possible source of ready energy for such events (Beers, 1956), and Allfrey, Mirsky and Osawa (1957) have shown that isolated nuclei possess an energy-generating system which might be involved in the processes of repair.

Steffensen's data make clear that the structural integrity of chromosomes is in some manner associated with the proper balance of divalent ions such as calcium and magnesium. Whether these metallic ions serve as links between blocks of DNA, as Mazia (1954) and Steffensen suggest, remains to be conclusively determined, but it has long been known that calcium, in particular, functions actively in the maintenance of membrane and gel stability. Calcium deficiency also leads to an increase in the X-ray sensitivity of chromosomes (Steffensen, 1957), while excesses of a variety of neutral salts lead to rather pronounced chromosomal damage, with heterochromatin the principal target (Gläss, 1956).

When we re-examine the data derived from studies with chemical mutagens several interesting facts emerge. The mutagenic action of $MnCl_2$ on cells of *E. coli*, for example, is governed by the treatment accorded the cells prior to mutagenic exposure (Demerec and Hansen, 1951). Unfortunately, we have little concrete knowledge of the state of chromatin in *E. coli,* but critical to the argument being developed is the fact that the mutagenicity of $MnCl_2$ is greater in resting than in growing cells, and is greatly increased by hypertonic solutions of several salts and sugars and decreased by the corresponding hypotonic solutions. It would therefore appear that circumstances favoring the dehydration of cells of *E. coli* also favor the mutagenicity of $MnCl_2$.

The action of a number of other chemical mutagens on plant cells is also suggestive. Among the more common mutagens are the diepoxides, cyanide, maleic hydrazide and beta-propiolactone. Each is highly preferential as to site of action on the chromosomes of *Vicia* (McLeish, 1953; Revell, 1953; Kihlman, 1956, 1957; Swanson and Merz, 1958), and when used at their lowest effective concentrations, heterochromatin is that portion of the chromatin which is preferentially affected. The parallel with the results of Gläss (1956) with metals is striking. It is consequently of significance to note that at the time of the most effective action of these mutagens in early interphase, heterochromatin is in a condensed state. By way of contrast, it can be pointed out that 8-ethoxycaffeine is an equally effective mutagen, and is highly specific in its action since it preferentially breaks the nucleolar organizer region of the long chromosomes of *Vicia,* but it acts in late interphase or early prophase after nuclear synthesis has occurred and when euchromatin

is beginning to condense (Kihlman, 1956). However, the nucleolar organizer region in *Vicia* exhibits no pronounced heterochromicity and the reason for the specificity of action of 8-ethoxycaffeine is not immediately evident.

Heterochromatin, in contrast to euchromatin, may be looked upon, then, as a preferentially dehydrated segment of a larger gel, lacking the water or the particular molecular organization which gives to euchromatin its comparatively greater stability. As the concentration of the mutagen is increased, and/or the time of exposure lengthened, the preferential action of the mutagens is blurred in that segments previously unaffected by low concentrations are now affected by high concentrations, but this is to be expected since the difference between euchromatin and heterochromatin is apparently one of degree rather than one of kind so far as is known. The stability of chromatin, so far as its reactivity with some mutagens is concerned, is further influenced by cellular metabolism at the time of exposure. Maleic hydrazide, for example, has its effectiveness modified by temperature, pH, O_2 level, and a variety of metabolic inhibitors (Kihlman, 1956). $MnCl_2$ behaves similarly as a mutagen (Demerec and Hanson, 1951). It has been customary to consider that these modifying factors are influencing the action of the mutagens. This is not necessarily the case, for there is no certainty that the responding structure, that is the chromatin, is unchanged. Indeed, when one considers the sensitivity of typical gels to environmental influences, it seems altogether reasonable to suppose that chromatin is capable of a wide variety of responses depending upon the mutagen employed and the cellular or nuclear circumstances at the time of exposure.

The role of calcium may again be mentioned in this context. The lampbrush chromosomes in the germinal vesicle of an amphibian are not clearly discernible until calcium is added to the suspending medium; the calcium-induced gelation is sudden and strikingly dramatic. It is worthy of emphasis, therefore, to point out that the action of beta-propiolactone is considerably enhanced by the addition of calcium salts at the time of exposure to the mutagen; magnesium salts are less effective (Swanson and Merz, 1958). Whether other chemical mutagens have their action similarly enhanced remains to be determined.

If, for the moment, it is assumed that the changing stability of chromatin is related, at least in part, to the changing organization and dynamics of a complex nucleoprotein gel, it should be possible to put it to a further test. We are immediately aware of the difficulties involved. Our criterion of stability, the frequency of aberrations, is a gross one, and we are not dealing directly with the immediate chemical reactions and interactions which, in the final analysis, govern the energy relations of the cell, the structural integrity of chromatin, and the effectiveness of the mutagenic agent employed. In the absence of sound biochemical and biophysical data which are applicable, the problem can only be approached indirectly. This we have attempted to do, the immediate rationale being that energy is necessary for the maintenance of the intact chromosome.

The root-tip cells of *Vicia* can withstand prolonged periods of anoxia. Short periods of anoxia have no lasting effects on chromatin; such treatments influence the degree of damage inflicted by X-rays, but there is no reason to believe that cellular energy is directly involved in the breakage process although it obviously is in rejoining. Anoxia periods of four hours or longer, however, lead to pronounced chromosomal damage, and with a continued depletion of oxygen and presumably of energy reserves, the frequency of aberrations increases until a

pronounced pycnosis and cell death intervenes (Merz, 1958). The distribution of breakage sites is not obviously preferential, suggesting that euchromatin and heterochromatin are affected to an equivalent degree.

Merz has also shown that the effect of anoxia is markedly accentuated by the simultaneous presence of NaF. This inhibitor is without effect when used in the presence of oxygen. Other metabolic inhibitors such as NaN_3, dinitrophenol, arsenite, and iodoacetate do not produce a similar effect, and it seems likely that NaF, in particular, is effective because it is specifically an inhibitor of anaerobic metabolism. It is of interest to note, however, that NaF is effective only after four hours of anoxia, and that longer periods of combined treatment did not appreciably increase the frequency of aberrations. It would appear, therefore, that four hours of anoxia and NaF is necessary in order to bring the root-tip cells of *Vicia* to a state of energy deprivation where chromatin instability in the form of fragmentation appears. This process is accentuated by the presence of NaF, but not by other inhibitors which have been used simply because anaerobic metabolism is effectively blocked, and the energy for chromatin maintenance is denied the cell. A suggestion of Beers (1956) may well be pertinent in this regard. He has proposed that RNA can act as a readily available source of energy in the cell. In terms of general cell maintenance, and in view of the ineffectiveness of anoxia for periods shorter than four hours, it might perhaps be more feasible to assume that DNA would act as a "last resort" source of energy, available only after other sources have been depleted. If DNA is an integral part of the chromosomal fabric, as is very likely, it is logical to assume that its breakdown would lead to the formation of aberrations. If true, it would then follow that those cells preparing to divide would be the ones preferentially affected. Merz has found this to be the case, since after the first burst of aberrant mitoses, the cells are almost entirely free of aberrations. Furthermore, if DNA were being utilized as an energy source, a continued DNA destruction would lead to cell death, and not simply the cessation of mitoses. Anoxic periods of more than four hours' duration lead to a greater and greater frequency of cell death, as Merz has shown.

It has been proposed that our understanding of the stability of the chromosome under normal as well as experimental conditions can be furthered by a consideration of the chromosome as a nucleoprotein gel whose state of hydration determines its sensitivity to X-rays and to chemical mutagens, and whose linear integrity is dependent upon a constant supply of energy. There are difficulties involved since this approach forces the cytologist to seek answers with the techniques of the colloid chemist, and there is an obvious meagerness of pertinent data. On the other hand, the data available are reasonably consistent with the point of view advanced and the approach offers a new vantage point from which the problem of chromatin stability can be re-examined and appraised.

REFERENCES

Allfrey, V., A. E. Mirsky, and S. Osawa. 1957. The nucleus and protein synthesis. *In* A Symposium on the Chemical Basis of Heredity, ed., W. D. McElroy and B. Glass, pp. 200–231. Baltimore: The Johns Hopkins Press.

Beers, R. F. Jr. 1956. Enzymic synthesis and properties of a polynucleotide from adenosine diphosphate. Nature, Lond. *177*: 790–791.

Demerec, M., and J. Hanson. 1951. Mutagenic action of manganous chloride. Sympos. Quant. Biol. *16*: 215–228.

EHRENBERG, L. 1955. Factors influencing radiation induced lethality, sterility, and mutations in barley. Hereditas, Lund. *41*: 123–146.

GLÄSS, E. 1956. Die Verteilung von Fragmentationen und achromatischen Stellen auf den Chromosomen von *Vicia faba* nach Behandlung mit Schwermetallsalzen. Chromosoma, Berl. *8*: 260–284.

KIHLMAN, B. A. 1956. Factors affecting the production of chromosome aberrations by chemicals. J. Biophys. Cytol. *2*: 543–555.

——— 1957. Experimentally induced chromosome aberrations in plants. I. The production of chromosome aberrations by cyanide and other heavy metal complexing agents. J. Biophys. Cytol. *3*: 363–380.

MCLEISH, J. 1953. The action of maleic hydrazide in *Vicia*. Heredity *6* (Suppl.): 125–148.

MAZIA, D. 1954. The particulate organization of the chromosome. Proc. Nat. Acad. Sc. U.S. *40*: 521–527.

MERZ, T. 1958. The effect of extended anaerobic treatments on the chromosomes of *Vicia faba*. Ph.D. thesis, The Johns Hopkins Univer.

REVELL, S. H. 1953. Chromosome breakage by X-rays and radiomimetic substances in *Vicia*. Heredity *6* (Suppl.): 107–124.

RIS, H., and A. E. MIRSKY. 1949. The state of the chromosomes in the interphase nucleus. J. Gen. Physiol. *32*: 489–502.

SPARROW, A. H. 1951. Radiation sensitivity of cells during mitotic and meiotic cycles with emphasis on possible cytochemical changes. Ann. N. York Acad. Sc. *51*: 1508–1540.

STEFFENSEN, D. 1957. Effects of various cation imbalances on the frequency of X-ray-induced chromosomal aberrations in *Tradescantia*. Genetics *42*: 239–252.

SWANSON, C. P., and T. MERZ. 1958. The action of beta-propiolactone on chromosomes. (in press).

WOLFF, S., and H. E. LUIPPOLD. 1955. Metabolism and chromosome-break rejoining. Science *122*: 231–232.

THE MUTATION THEORY RE-EXAMINED

H. J. Muller[1]

The Uniqueness of the Mutable Material

A MUTATION differs from other natural changes in that it can undergo replication, that is, reproduction. The mutation is reproduced, of course, in the replication of the genic material in which it originally occurred. In other words, when this material is replicated, it includes in the process its own changes, at least some kinds of changes. A virtually unlimited number of diverse replicable changes, including concretional ones ("repeats"), can be accumulated in the genic material. Thus, in the course of ages, the material will inevitably have amassed an increasing collection of those changes which proved successful, that is, changes serviceable to further replication of the material. As a result of this accumulation, the genic material and its associated products have become so highly organized, in varied lines, that we set the resulting systems of matter apart from everything else known in a category which we designate as "living."

In presenting this argument at Toronto in 1921, I left open the question of whether the genic material itself contains the essentials of the replicational mechanism or merely serves as a kind of stencil that fits into a mimeographing mechanism present in the protoplasm. However, in a paper "The gene as the basis of life," given five years later to the International Congress of Plant Sciences in Ithaca, I presented reasons for concluding that the genic material itself contains the replicational mechanism, and that protoplasm has gradually arisen, secondarily, as a series of useful by-products, resulting from the activities of the naturally selected mutant genes.

The main argument for this hypothesis was that non-genic material, that is, material without the faculty of alterable replicability, would have had to have a highly elaborate organization if it had embodied the potential ability to replicate genic material according to the pattern of that material, when, later, that material was presented to it, and if, *in addition*, it had contained (as would have to be postulated) the means of reproducing its own structure. The mathematician Von Neumann in 1946 conceived that a machine might be built which could both construct other machines like itself and also replicate modifiable blueprints or coded records.

The difficulty with this protoplasm-first hypothesis is that the complicated organization required would have had to fall together through an unselected concatenation of series of accidents. It could not have been evolved through many small, naturally selected steps because, being itself non-genic, it would have lacked, in its intermediate stages, the faculty of replicating its own alterations. Thus we return to our conclusion that the first step in multiplication and growth was genic multiplication—the type of replication that includes a replication of alterations in the pattern. That is, the first multiplying material was the genic material or protogene.

[1]Department of Zoology, Indiana University, Bloomington, Ind., U.S.A. (Contribution 573).

At the time this conclusion was drawn it was already certain that the nuclear chromosomes form the main seat of the genic material, with the chloroplasts and their relatives forming a secondary seat in plants. However, it could not yet be decided whether the protein, the nucleic acid, or both together, or some unknown material present in smaller amounts and associated with them, constituted the genic material itself. As we all know, the brilliant work of a series of investigators since that time, including those working on diverse viruses and on so-called transforming substances of bacteria, have narrowed down the property of alterable replicability first to the nucleoprotein and then to the nucleic acid alone. Meanwhile, the epochal theory of Watson and Crick, corroborated by still more recent studies, has depicted the structure of the genic nucleic acid and has shown how this structure replicates itself by rearranging scattered particles through unions of complementaries. On this model, it is also evident why even an alteration in the linear pattern is copied in the replication process.

Benzer's work has brought forward strong evidence that in phage the mutations most often consist of substitutions of individual nucleotides or nucleotide pairs. And his evidence from the groupings of the nucleotides concerned, in mutations involving more of them than a pair, has shown that they are linearly arranged down to the level of the individual pair, as on the Watson-Crick model. That is, there is room here only for a double chain, not a polytene or ribbon-like one. Moreover, since the mutations in the past evolution of the phage chromosome left it with its standard four types of nucleotides, arranged in the given way, it can be inferred that the point substitutions are ordinarily limited to these four components. Finally, the *en bloc* changes also must have preserved the fundamental structure, and must therefore have consisted, like the large-scale structural changes of chromosomes known in higher forms, of linear deletions, inversions, and insertions of segments comprising several or many of these same nucleotides.

That these conclusions based on phage may be extended to *Escherichia coli* is made highly probable by the recent work of Adams and Luria. For this work indicates that pieces of phage chromatin and bacterial chromatin may undergo a type of interchange with one another so that, when a part of the bacterial chromosome replica is carried by the phage into another bacterium, the phage replica lacks some portion of its own chromatin. This result, then, argues for a single-file, non-ribbon-like arrangement of nucleotide pairs in the bacterial chromatin also. Since the line between bacteria, fungi, and algae is by no means sharp, it is tempting to attribute the same non-multiple constitution to the chromosomes of higher organisms as well.

But there is also evidence for the same conclusion from higher organisms themselves. This consists, for one thing, in the fact (discussed in my earlier papers) that mutant genes arising in a given cell are ordinarily distributed to all or approximately one coherent half of the descendant somatic and/or germinal tissue, without undergoing further mitotic segregation. Another line of evidence lies in the difficulty to be encountered on the ribbon hypothesis, in getting all chromosome strands, especially those of ring chromosomes, accurately matched up in their unions in successive acts of crossing-over, of restitution after breakage, and of structural rearrangement.

Changes Caused by Cross-influences between Strands

The Watson-Crick doubleness provides a ready interpretation of the fractional expression of so many of the gene mutations that are found after exposure of

spermatozoa to ionizing radiation and of pollen to ultraviolet. A recent finding by Carlson of an inherited double-fractional mutation, involving one subgene of the complex dumpy locus in one "half" of the body and a different subgene of it in the other half, is a striking case in point.

The known doubleness makes it harder, however, to explain the other cases, those in which a seeming gene mutation arising from a treated gamete appears to involve the entire offspring. Perhaps, as in D. Lewis's studies, there is an unstable period after the origination of a mutation, in which it may revert back to normal. If in his cases this represents the period antcedent to the separation of the Watson-Crick strands there might at this time be a conflict of influences between the two non-matching members of the asymmetrically affected pair of nucleotides, in which sometimes the mutated and sometimes the unchanged member succeeded in getting a complementary nucleotide substituted in the place of its misfitting partner.

The assumption of this type of cross-influence is difficult to reconcile with the findings of Levinthal in phage, based on tracer studies. These show that in this organism the regular method of recombination between two conjugated chromosomes of different origin is by the production of daughter nucleotide-chains each of which is a complementary replica of a chain of one parent chromosome up to a given switch-point, and of the corresponding chain of the other parent chromosome beyond that point. In view of the interchangeability of phage and bacterial chromatin, this process may occur in bacteria also. Now it is to be observed that each so-called "daughter" chromosome has the usual Watson-Crick doubleness, in which one chain is an unchanged parental one and its partner is newly formed. Hence the "daughter" chromosome that was produced by recombination must have had non-matching (non-complementary) members of those pairs of nucleotides beyond the switch-point in respect to which the two original chromosomes had disagreed. Yet the regularity of the subsequent segregation ratios and linkage relations shows that the improperly matched chains succeeded in surviving and replicating as such. In fact, this holds true even in *en bloc* mutations involving deletions or inversions, and therefore non-matching, of whole groups of nucleotides. It follows not only that virtually single nucleotide-chains can undergo replication (by complement formation), but also that non-complementarity of nucleotides, under certain circumstances at any rate, does *not* result in one of the two non-complemented nucleotides becoming replaced by a complementary one.

That a similar switching of parentage between homologous chromosomes, sometimes called "copy-choice," can sometimes occur in forms beyond the bacteria, has been shown by the cases called "conversion" that have been observed by Lindegren in yeast, by Mitchell in *Neurospora*, and possibly by others in *Drosophila*. Some of these cases have been found to involve exchanges of chromatid parentage between subgenes of the same locus complex. In such cases, unlike those of true crossing-over, there is a tendency for one switch-point to be accompanied by another one nearby ("negative interference"). In these cases, however, there is no evidence that the cross-influences caused changes in the composition of pre-existing chromosome strands or that any real transformation other than linear recombination was involved.

At the same time, it should be emphasized that *Drosophila* studies of the results from attached X-chromosomes and from rings, as well as other kinds of tetrad analyses, have proved conclusively that here crossing-over ordinarily involves a physical breakage and exchange of pre-existing strands, unlike the process postulated by Belling or that demonstrated in phage.

In contrast to the types of phenomena thus far reviewed stands a small group of cases of what Brink has called "paramutation." Here cross-influences exerted between differing homologues during meiotic replication seem to result in actual transformations, as in Brink's results with stippled maize, Demerec's findings on reddish alpha in *Drosophila virilis*, and the cross-induced breakage of chromosomes found by Sandler, Hiraizumi, and Sandler, working with Crow on *Drosophila melanogaster*. In some of these cases the ratios show that here it is not merely a matter of exchanged parentage, even though some sort of recombinational effect may have been involved.

Despite these remarkable cases, the old evidences and arguments against the occurrence of a mixing or contamination process between alleles remain valid, as applied to inheritance in general. Certainly the great majority of gene changes originate by no such process. Moreover, a mass of experience with mutational effects, including broken chromosomes, indicates that in higher forms such phenomena as transduction, conversion, and paramutation cannot be achieved by such crude means as the injection of foreign DNA, treatment with antigens or antibodies, or tissue transplantation.

Is Gene Mutation a Valid Concept?

The old representations of genes as beads on strings were not intended to show their actual shape or inner structure, but only their linear arrangement. It was mentioned in some of the early papers that their structure must be very complex, reflecting a long evolution. At the same time, arguments were presented by Altenburg and myself against the genes being composed, like gross objects, of a collection of identical molecules, and this conclusion has been borne out by the modern findings concerning the genic DNA.

Direct evidence of gene complexity was found, in the twenties, in multiple alleles, such as those of the dumpy, scute, and white loci, giving varying patterns of pleiotropic effects. The evidence, in the twenties and thirties, for the existence of small adjacent repeats in *Drosophila*, both already established ones as in the case of scute and achaete or the sperm motility genes of the Y, and newly arisen ones as in Bar and Abruptex, was used to illustrate not only how the genome can increase but also the complex potentialities of the original gene that had undergone both repeat formation and (if long established) further differentiation.

Drosophila position-effect studies of the mid-thirties showed, further, that regions of widely separate origin can, when brought close together, participate through local interactions in the manifestation of allelically expressed characters. It followed both from these and from the repeat studies that chromosome breakage and crossing-over can occur between parts of a small chromosome region concerned with a common function.

E. B. Lewis's and other more recent studies on pseudo-alleles have abundantly illustrated the divisibility of long-established functional regions by crossing-over. In fact such cases now appear so typical that it seems advisable to drop the prefix "pseudo" for them. Nevertheless, the sub-regions thus distinguished are of inordinately greater physical length than those studied by Benzer. Moreover, there are indications (not yet proof) that physical crossing-over in higher organisms, unlike parentage-switching (copy-choice) in phage, may be restricted entirely or mainly to given nodes, defining internodes or subgenes comprising thousands of nucleotides.

Our criteria for whether two mutants of *Drosophila* represent changes of the

same gene do not entirely coincide with Benzer's criterion for a "cistron." Not only are there occasional synergistic interactions in organisms simultaneously heterozygous for entirely separate genes, individually recessive for the given effect, as happens with truncate or dumpy in connection with given intensifiers or with forked in connection with Belgovsky's "If," but there are, conversely, numerous instances of phenotypically normal or nearly normal heterozygotes obtained by crossing two recessives, mutants of the same region, even though both of these mutants act as alleles or pseudo-alleles of other "point-mutational" members of that series. In the better known of these heterozygotes, such as vortex and oblique or scute 5 and scute 6, this phenomenon is readily understandable, since the two so-called "complementary" alleles affect different characters. However, in a number of cases, several of which (involving lozenge, facet, etc.) I reported at the 1932 Congress, the two complementary mutants have, separately, much the same phenotypic expression. Certainly they belong to the same functional genetic region or gene, yet by definition they do not belong to the same cistron. The most instructive example of this kind is one recently found by Carlson in the dumpy (truncate) series. Here two recessive lethals occupying different sub-loci are not lethal in compound (mutually heterozygous in trans relation) with each other, yet each is lethal in compound with any one of several other recessive lethals of the series which occupy still other (separable) sub-loci.

That the seemingly clear-cut cistron delimitations of phage would be oversimplifications for *Drosophila* is further shown by our diverse position-effect cases in which a gene from a totally different euchromatic region, surely subserving a different function, nevertheless results in an allelically acting effect involving a given region, when a structural change places it near that region. Thereby, in fact, two functionally different regions come to overlap one another. Moreover, other long-known position-effect evidence (such as that from double Bar) shows that one functional region, manifesting a cistron-like difference between trans and cis phenotypes, may be in two or more sections, separated by a small stretch of interstitial genetic material having other functions.

Despite this vagueness of delimitation of the gene, there is a real differentiation in function between chromosome regions. And just as the terms atom and species retain their usefulness despite the proof that the things they stand for can split, unite, or intergrade, and just as we do not discard the term "origin of species" or deny the existence of *Homo sapiens* because of such reservations, so too with the gene. We are glad, however, that the analysis of these realities is proceeding further.

We raised the question in the mid-thirties, following our findings of minute linear rearrangements, whether all seeming gene mutations might not be such changes, on a still smaller scale. It was pointed out, however, that if this were true the changes of evolutionary importance must lie within the gene as then conceived, in order to allow the versatility of change necessary for extensive evolution. It is clear from recent work that this qualification was correct, inasmuch as *Drosophila* mutations have been tracked to within the subgene, and phage mutations followed virtually to the nucleotide level.

There is reason to suspect, however, that multigenic structural changes in higher organisms may usually result from breaks at nodes between genes or subgenes, while those within the gene affect a different type of bond. Why otherwise should practically all tested translocations produced in mice and in higher plants appear normal when homozygous? Again, if intragenic mutations result from chromosome breaks of the same kind as those that give multigenic rearrangements, why should

the frequency of gene mutations found after irradiation be as high for the ring chromosome of *Drosophila*, that is so readily lost by imperfect restitution after breakage, as it is for non-rings—a result first found by Offermann, and recently confirmed in careful tests by Oster? Evidently, then, if the gene mutations do involve breaks, they are not those full-fledged breaks that can be followed by demonstrable structural change or by a failure to restitute properly.

This argument is not invalidated by the fact that the mutations here studied were induced by radiation. For neither the gene mutations nor the structural changes thus induced give evidence of being different in their essential nature from spontaneously arising ones. Some of them have been shown in experiments by Oster, Muller, Green, and Carlson on the forked and dumpy regions of *Drosophila*, and by de Serres on the adenineless region of *Neurospora*, to be subgene mutations, including partial and seemingly complete back-mutations, of sensibly the same types as previously known spontaneous ones. Although we have never claimed that back-mutations constitute structurally precise reversals, their production does remove the earlier suspicion that only losses are produced by ionizing radiation. Furthermore, as Caspar and Singleton have shown, ionizing radiation in maize, when applied at an ordinary interphase stage, results in gametophytically viable mutations resembling the spontaneous ones although, when applied to mature and maturing gametes, the radiation induces such a flood of multigenic rearrangements as to swamp these out. It is true that in this paper I am not concerning myself, except peripherally, with multigenic rearrangements; nevertheless I bring all these results forward here because they indicate that, in higher organisms, such changes are distinguished from "gene mutations" by more than just the scale of magnitude involved.

The Instigation of Mutation

In considering the mechanism of the mutational process we must still recognize the importance of randomly directed movements of particles—Troland's "molecular chaos." This "chaos" is evidenced by the pointwise incidence of mutation which involves one gene in a diploid cell while leaving its counterpart nearby unaltered. It is also evidenced by the generalized rise in mutation rate caused by rise of temperature or by irradiation. Yet besides the frequency and the amount of energy of the encounters involved, many other factors play a role. This is shown, for one thing, by the influence of definite mutagens of strong effect, as found first by Auerbach and Robson, and by the lesser influence, both plus and minus, on the mutation frequency, of other known chemicals, as shown in the work of Novick and Szilard, Demerec, and many others. It is also shown by the decided influence exerted by genetic composition and by physiological state. It is obvious that the likelihood of mutation in a given gene is bound up with the whole complex of metabolism, even though at the same time the precise incidence of the individual mutations depends upon accidents of the ultramicroscopic configurations.

It is not surprising, then, that the groups associated with Giles, Kølmarck and Westergaard, Newcombe, Bryson, Demerec, and others working on micro-organisms, and Gustafsson, Kaplan, Ehrenberg, and others on higher plants, have found marked differences between mutagens in the relative incidence of mutations produced by them in different genes, including different alleles of the same locus, and that these genes and alleles also differ in their spontaneous frequencies and spectra. In most of the organisms tested the differences are seldom absolute, in that most mutagens tried affect most genes to some extent. Demerec, however, has here

reported to us a high proportion of mutagen-stable alleles in *E. coli*, while Benzer finds that in phage on the nucleotide level there is a clear-cut disjunction between some mutagens.

In *Drosophila* and mice, by contrast, the spontaneous and radiation-induced spectra have proved remarkably similar. Sobels and the Altenburgs have extended this similarity to some chemical mutagens, at least so far as what might be called classical mutants are concerned. The Fahmys report, however, that certain chemically induced mutants of theirs fail to respond so readily to radiation. Tests of the same mutants by others are here in order. But no matter how this question may be decided, the difficult problem remains, why should the radiation and the spontaneous spectra of mutation be so much more like one another in *Drosophila*, and probably also in mice, than they are in the micro-organisms studied? Are these animals' genes so much more complex as to make more of the mutations in the same gene and subgene indistinguishable from one another? Or is there a less effective differentiation, along the chromosomes of the animals as compared with the micro-organisms, or extra-genic substances that act as intermediaries in mutation?

If the action of mutagens is directly on the nucleotides one would hardly expect mutagens to be differentiated by marked specificities except at the nucleotide (as contrasted with the gene or subgene) level. If, however, a mutagen meets a linearly differentiated barrier of highly organized extra-genic material which it must usually first penetrate and/or change, then such specificities are readily understandable. That the gene can be changed secondarily, as a result of a violent chemical disturbance in non-genic material closely associated with it, has been indicated by the experiment of Hungate and Mannell on *Neurospora*, in which radioactive sulfur incorporated in the chromosome appeared to induce mutations not only by way of the ionization tracks arising from it but also by the transmutation of its atomic nuclei. If substantiated, this finding would mean that the alteration of the protein which contained this sulfur caused, in turn, a mutation of the gene proper, that is, the DNA. The result thus seems to lend support to our interpretation that mutagens may sometimes act indirectly, via the chromosomal protein.

In radiation mutagenesis there can, of course, be several intermediate substances involved, as shown first by Stone and Wyss's demonstration of the role of peroxides in some of the mutagenic action of ultraviolet on bacteria. It is true, however, that in higher organisms the mutagenic pathways are usually much shorter than those studied by Stone and Wyss. This is illustrated clearly, for chromosome breakage, by the linear relation between the radiation dose and the frequency of two-break translocations when dense ionization tracks are used, as compared with the 3/2 power or square relation found with X- and gamma-irradiation. It is further indicated by the continued increase in the effectiveness ("RBE") of tracks in breaking chromatids, even as their ion-density is raised far beyond the point where ordinary chemical effects have reached a plateau. This result seems best interpreted as meaning that both strands of the double DNA chain, and possibly the protein, must usually be broken, *and* that the effective ionizations must arise near the sites to be broken. However, it does not follow that the DNA itself must usually be directly ionized although this must certainly happen sometimes.

There is evidence that in addition to the steps referred to above, which are antecedent to the change in the chromosome, there are often later steps, necessary for the finalization or stabilization of a gene mutation. Such evidence was first found in *Aspergillus* by Hollaender, Stapleton *et al.*, and later in *E. coli* by Witkin. Such evidence also lies in Auerbach's finding that the frequency of recessive lethals

induced by irradiating the spermatozoa within *Drosophila* males is reduced if the sperm are not soon afterwards ejaculated. The same conclusion follows from Sobels' recent finding that the lethal frequency is increased by cyanide given to *Drosophila* spermatids after their irradiation. Are these after-effects expressions of a temporarily labile physicochemical configuration peculiar to the affected component of the chromosome (whether at this stage it be protein or nucleotide), or do they represent a period prior to the establishment, at the given point in the chromosome, of matching alterations in both or all components: the two nucleotides and their attached amino acid?

However these questions may some day be answered, it is evident that gene stability depends on many factors. As I pointed out in 1918, there must have been a natural selection favoring high stability: both stability of the genes themselves, giving them resistance to mutational changes, and stability of the reactions whereby the genes produce the normal type. Both these kinds of stability might be termed forms of homeostasis, had not that term been so misused recently that it is now misleading. At any rate, it is evident that present-day forms of life embody a high degree of organization directed toward the maintenance of the stability of the normal type, and that this organization has been attained by the prolonged selection of countless steps. By tending to be subversive of this organization, both genetic and environmental changes of unusual kind or degree would be much more likely to increase than to reduce the mutation rate, and also more likely to increase than to reduce the variance of gene expression. In fact, this has been found to be true.

As Sturtevant once pointed out, the selective circumstances in most organisms may be expected to give them a somewhat lower than optimal mutation frequency, since the detrimental effects of mutation are usually more immediate and continuous than the beneficial effects. On the other hand, in some organisms, especially those having long lives and low reproductive pressure, the optimal mutation rate per unit of time must be unusually low, and these may be unable to attain even so low a mutation rate as would be optimal for them.

However, there is a circumstance that helps long-lived organisms to attain a much lower mutation rate than their life span might lead one to expect. This lies in the fact that in shorter-lived species, as exemplified in *Drosophila* at any rate, so large a proportion of the mutations are concentrated into one group of stages, the peri-fertilization stages of post-gonial germ cells and early cleavage. These stages do not occupy nearly as much time, in proportion to the total life span, in long-lived as in short-lived species. That the great majority of spontaneous mutations in *Drosophila* occur in these stages was found in my experiments on aging reported in 1946, and has been confirmed in the recent work by Schalet and by Hildreth and Carson.

For radiation mutations, many workers have found the post-gonial stages of germ cells of both sexes to be especially mutable, in diverse animals, and for most chemical mutagens tried this has also proved to be true. Now, in order to determine whether the high spontaneous mutation rate of early zygote stages is paralleled by a high radiation mutability, our Indiana group during the past year decided to put these stages to the test. The tests, some of which have just been completed by Oster, show a low induced mutability some ten minutes after egg laying, followed some fifteen minutes later (during the period of rapid zygotic division) by an extremely high induced mutability (twenty-one recessive lethals in 345 X-chromosomes treated with 800 r, or 6.1 per cent). This is as high or higher than the rate of the most mutable (for ionizing radiation) stage previously known, that of spermatids. Inde-

pendently, Ulrich has simultaneously obtained results of the same character, which he has reported at Burlington and at the present Congress.

These results, taken together, lend support to the contention that long-lived organisms, even without an increased selection for a lower time-rate of mutation, would not have a mutation rate per generation nearly as much higher than that of short-lived organisms as a proportionality of mutation frequency to generation length would give them. They must however be subject to increased selection pressure in the same direction. Thus the per-generation similarity of the spontaneous frequencies found in such divergent types as men, mice, and flies becomes more understandable.

It might be imagined that the higher mutation frequency of peri-fertilization stages is merely a consequence of most mutations consisting of mistakes in genic replication. However, gonia reproduce themselves more promptly, after irradiation, than do the spermatids of pupae. Moreover, the effects found in cleavage stages are too great to be explained on this ground alone. Probably peculiarities of the condensed condition of chromosomes somehow make them more mutable. But whether most mutations are not finalized until replication occurs, and whether they then reach realization chiefly in the newly formed nucleotide-chains, are questions still awaiting solution. Whatever the answers may be, many of the mutations induced in gametes by ionizing radiation do involve changes in the original as well as in the filial strands, as shown by their whole-body manifestation.

Mutational Expressions

Time does not permit more than brief reference to some of the fascinating problems concerning mutational expressions. One question is how the enormous subgenes and genes, in higher organisms, co-operate locally near their chromosome site in fashioning the common product of a given locus. Despite Demerec's evidence that in bacteria some closely linked genes pass their products along unidirectionally, conveyor-belt fashion, this is unlikely to be the rule in *Drosophila*, since here some euchromatic position effects have long been known to be exerted across intervening genes that have unrelated functions. In *Drosophila*, moreover, the position effects of heterochromatin, at least, can be extended in either direction along a chromosome. Again, in a case of E. B. Lewis's, involving the bithorax complex, a position effect is exerted between a subgene in one chromosome and a different subgene in the homologous chromosome. Evidently the product of one of these subgenes can be acted upon to some extent by another subgene or its product, complementarily, without these subgenes having to be in any precise position with respect to each other so long as they are fairly near together. The nature of the product itself, at the given step, must in such a case determine what nearby substance is to act upon it, somewhat as must happen in the protoplasm in general in non-positional interaction.

So intricate and highly organized as a result of past selection is the web of gene-product interactions which constitutes the workings of protoplasm that it should be quite evident *a priori* that blind changes in the genes would usually be harmful, and that the more drastic ones would tend to be more harmful. This principle is today far better established experimentally than when it was put forward by this writer in 1918, and again in my paper of 1921 entitled "Mutation," although there have at all times been new-fledged results, later disproved, that claimed to overthrow it. During recent years, more especially, there has been an enormous pressure from outside genetics that has manifested itself in diverse forms of opposition to this

principle, with the underlying motive of denying or minimizing the harmful genetic effects of radiation. Ultimately, however, this semi-political dispute should result in the attainment of more exact knowledge on the subject.

Another principle to which attention had been called in my articles of 1918 and 1921, namely, that mutant genes tend to have much less dominance than their "normal" progenitors, has been seen on further consideration to be a corollary of their detrimental nature. For, as was pointed out at the Ithaca Congress by both Plunkett and myself, this dominance has resulted from a long selection for stability of expression that has brought normal genes to so high a level of effectiveness that disturbing factors exert little influence on the effect which these genes produce. Mutant genes, on the other hand, being mainly disorganizational, usually have a lower effectiveness and therefore a more variable expression, while in the heterozygote the one dose of normal is usually effective enough to give what seems to be a normal phenotype. However, more delicate tests, such as those involved in dosage compensation studies or in chemical extractions, show that the heterozygote is usually to some degree intermediate after all. It has followed further from dosage compensation that even this imperceptible deviation from normal has usually been enough to cause the elimination of the mutant gene from the population while it was still in the heterozygous condition.

Unfortunately, we cannot here give due consideration to the recent claims, harking back to pre-Mendelian views, that heterozygosity in itself tends to be beneficial. It should, however, be pointed out that the evidence from inversions in *Drosophila* is quite beside the point since they carry with them intact multigenic complexes established by selection. A situation comparable to this cannot exist in most organisms since crossing-over in the male (unlike that in the female), when it occurs within heterozygous inversions, results in meiotic bridges that lower the reproductive potential and thus eliminate the inversions before they can accumulate complexes that possess a net advantage when heterozygous.

As for evidence of benefits derived from heterozygosity at individual loci, the critical cases in point are so rare and special (although they tend to make themselves conspicuous!) as almost to prove that they are contrary to the rule. Certainly, the previously mentioned complementarity that is sometimes found between two mutant alleles or pseudo-alleles of the same chromosome region afford no valid argument in this connection, inasmuch as the normal has in these cases proved superior to both mutants. Moreover, even if this had not been so, the superior features of both alleles should usually be combinable within the same chromosome by further mutation and/or by crossing-over. As for those examples, known in man, in which given recessive anomalies, such as cystic fibrosis, occur at too high a frequency to be readily explained as a result of the mutation pressure originating at a given locus, it is to be observed that some of these may well represent collections of genetically heterogeneous, but phenotypically similar phenotypes which can result from mutation at any one of many loci, as is true of minute bristles and rough eyes in *Drosophila* and of some frequently encountered abnormalities in mammals (such as the vestibular dysfunctions cited in Nachtsheim's paper at the present Congress).

To be sure, the blood antigens present undoubted examples of selection for heterogeneity (although not necessarily for heterozygosity *per se*) that must be of some permanent advantage, of a kind that we do not yet understand. However, as I have argued in my paper "Our load of mutations," most *bona fide* cases of heterozygously superior loci, such as that for sickle cell anemia, are to be regarded as temporary makeshifts that arose in the stress of comparatively rapid evolutionary

flux and that are due to be rectified ultimately, when a longer-term natural selection repairs its short-term imperfections and miscarriages. As Crow has shown, the data from consanguinity studies in man allow the existence of relatively few examples in which the heterozygote is superior to both homozygotes of an allelic pair. And the more mutually heterotic alleles of a locus and the more such loci we postulate, the deeper is the water we get into in accounting for a favorable over-all balance. Kimura has recently made an astute analysis of the statistical and other difficulties encountered by the hypothesis of so-called "heterotic loci." We hope that his paper will soon be published.

Before leaving this subject it should be observed that even if heterozygosity did confer a *per se* advantage it would be expected that each species having large effective population numbers would in consequence of natural selection have tended to increase its degree of heterozygosity to nearly its optimum level. Thus, any further increase in heterozygosity, such as would be brought about by the application of mutagens, would not be to the species' advantage.

Several other arguments besides that of "homeostasis" are being put forward nowadays in the attempt to defend the production of an increase in genetic variation. One is that most mutations are small or even subliminal, and that these are about as likely to be helpful as harmful, or more likely, when heterozygous. A logical error is however involved in thinking that a small deviation from the norm will not usually be detrimental, at least in a small way. Moreover, as I pointed out in discussions of some forty years ago, although there are reasons for inferring that far more mutations are subliminal than visible, even the subliminal mutations cannot be inordinately much more frequent than the ones we detect. For, if they were, a genetic drift would tend to occur that was more rapid than observed. At the same time, as we can now see, the checking of this drift by selection would require more elimination than some species (such as man) could afford to undergo.

Another popular misconception, endorsing as great a variance as can be achieved, is that the possession of many heterozygous mutant genes by a diploid population gives it a more useful store of variability in case of need than would be available to a haploid population. A little analysis shows, however, that the diploid accumulates mutant genes only to the point where it has the same amount of phenotypic variance per genome that it would have if it were haploid, and that, if conditions changed, no more material would be available for selection in the diploid than in the haploid. Because so much of the diploid's variance is under cover, however, selection would move the diploid's mean much more slowly than that of the haploid, that is, it would achieve its result less readily. These considerations apply as well to short-term or cyclic genetic adjustments as to long-term evolution.

Still another type of genetic diversity is that which is supposed to be sustained by differences in the type of selection acting on a species in different places, times, or situations. Except for cases of local isolation, however, such differences do not ordinarily tend to result in an actively maintained genetic diversity. They result in compromise, in phenotypic versatility, and in the genetically wider spread that any relaxation of selection would allow mutation pressure to bring about. The result is different if the individuals concerned engage in assortative mating, either directly or as a result of choosing the conditions to which they are better fitted and thereby selectively encountering and mating with their likes. Thus more or less differentiated endogamic castes could be established. But in most species such situations probably do not exist with much strength. Genetic diversity is also maintained by selection in the rare cases of characteristics that are advantageous when below a certain level

of frequency but disadvantageous when more abundant. Examples of this situation are furnished by the diversity of markings on grasshoppers that *hinders* the recognition of their species by predators, and by the similar diversity in some fishes that *favors* the recognition of the individual by his potential mates and associates. However, after allowance has been made for such circumstances, the selectional factors that are effective in making for genetic diversity within a local population remain meager for most groups.

That even human cultures have not yet, through any of these modes of operation of selection, resulted in much increase in genetic diversity, at least so far as skeletal characters may be used as an index, is indicated by measurements made on wild gibbons by A. H. Schultz (Am. J. Phys. Anthrop. 1944). These data show that local populations of the uncultured gibbons have an amount of variance similar to that in local populations of modern man!

It is thereby again indicated that even our own array of mutations are—contrary to implications in the 1958 United Nations report—mostly the result of a mutation pressure that acted against selection, not the result of a selection that tended to preserve them. It is nevertheless true that our modern conditions do make certain types of genetic diversity useful and desirable. They also make desirable the attainment of higher mean levels, especially in the genetic basis of mental characters. But the way to achieve and maintain more salutary diversities and more salutary means does not lie in any generalized increase in mutation rate, such as any known mutagen would induce. *Any old mutation will not do.*

The only method yet known for preserving or advancing an already intricate, genetically based organization is the method of selection, natural or artificial. That natural selection alone is not to be trusted indefinitely is attested to by the wreckage of species left behind us in the evolutionary struggle. Moreover, we are already interfering seriously with those processes of natural selection that gave us the genetic gifts we have. Whether we can hereafter make artificial selection saner and more reliable than natural selection, and whether we can raise man far enough *without* selection to enable him to operate selection soundly: those are our greatest human problems and challenges. In pursuing them we must, among other things, learn to know our mutations, and to make use of them. Meanwhile, it should be recognized that the making of decisions in this field will require the most exacting exercise of our judiciousness, our humanistic qualities, our wisdom, and that the implementation of these judgments will demand radical re-education and consummate statesmanship.

SYMPOSIUM

PHYSIOLOGICAL GENETICS

1. C. Pavan. Morphological and Physiological Aspects of Chromosomal Activities
2. Ernst Hadorn. Contribution to the Physiological and Biochemical Genetics of Pteridines and Pigments in Insects
3. J. R. S. Fincham. The Role of Chromosomal Loci in Enzyme Formation
4. Ray D. Owen. Immunogenetics
5. P. Michaelis. Cytoplasmic Inheritance and the Segregation of Plasmagenes

MORPHOLOGICAL AND PHYSIOLOGICAL ASPECTS OF CHROMOSOMAL ACTIVITIES

C. Pavan[1]

DURING THE DEVELOPMENT of multicellular organisms there occur certain cellular changes which are conditioned either by differentiation of the cytoplasm or by differentiation of the nucleus. Until very recently it was generally thought that the former would be very common while the latter would be rare. The usual and most probable view was that cellular differentiation was cytoplasmic and would, therefore, persist and be transmitted to daughter cells by cytoplasmic heredity (Wright, 1941).

As pointed out by Mather (1948), the special changes in the nucleus during differentiation appear to be of three main kinds: changes of elimination, replication, and manifestation. The equational character of mitosis and the general agreement among cytologists concerning the simultaneity of the duplication of the gene and the duplication of the chromosomes were strong arguments against any other changes of the nucleus.

The finding that the amount of DNA was constant in cells of various tissues of an individual was another good argument for the general theory that there was a genic equivalence in different cells of an organism (Boivin *et al.,* 1948; Mirsky and Ris, 1949; Swift, 1950). This constancy of DNA was not accepted by some cytologists (Brachet, 1957) who found that the amount of DNA varied in individual nuclei of the same organism. Lison and Pasteels (1951) advanced the theory that this variation of DNA could be related to the process of differentiation, and although they believed that there was a constant median value in different nuclei of the same organism, they assumed a variation in individual nuclei which could not be explained by the addition or subtraction of an entire genome (Lison and Valeri, 1956). This theory was accepted by Moore (1952, 1957) who attempted to use it to explain the variability in DNA which she found in her experiments with embryos of *Rana pipiens*: "It may well be that during differentiation DNA may be produced in variable amounts at different regions of the chromatin." This would be a type of variability not related to polyploidy or deletion. A certain number of other publications, not only on embryonic development but also on adult tissues, disagree with the assumption that the quantity of DNA is constant in all cells of a tissue (see Brachet, 1957).

On the other hand, the belief that an obligatory chromosomal division will always occur when there is a gene division was questioned in some very recent work which suggests a new kind of nuclear differentiation (Breuer and Pavan, 1952, 1954, 1955; Pavan and Breuer, 1955; Ficq and Pavan, 1957; Rudkin and Corlette, 1957; Stich and Naylor, 1958 a, b). Finally, the work of Briggs and King on transplanting nuclei of amphibian eggs showed that the nucleus undergoes some changes during differentiation which are not related to polyploidy or loss of chromatic material. The purpose of the present contribution is to discuss some data on the morphology

[1]Department of Biology, Universidade de São Paulo, Brazil.

and physiology of polytene chromosomes, and to show how these studies may shed light on the problems of nuclear physiology and nuclear differentiation of a kind not related to the one reviewed by Mather and mentioned above.

Giant Chromosomes

In general, the chromosomes are not sufficiently distinguishable as individual entities except during nuclear division, when very special changes occur in their structure. An exception to this rule is the polytene chromosomes of the Diptera. The lampbrush chromosomes of vertebrate oocytes also show some special features not common to other meiotic chromosomes. These two types of chromosomes show some similar features during their metabolic changes which were considered until recently as identical, but recent results (Ficq, Pavan, and Brachet, 1958) show that in reality they may be fundamentally different, as we will discuss later.

The polytene chromosomes are special structures found in the nuclei of cells of different tissues (Trager, 1935; Makino, 1938; Cooper, 1938; Beermann, 1950; Pavan and Breuer, 1952) of several families of Diptera (Mainx, 1949). In all known species the salivary gland cells are the ones where these chromosomes attain the maximum size and thus permit more detailed analysis. These chromosomes may be over a hundred times larger than the mitotic chromosomes; they present a sequence of dark-staining bands separated by a non-staining interband material.

Passing in review the various theories of the structure of the polytene chromosomes, one may see that the main problems involved are: (*a*) the cause of the great increase in width and in length of these chromosomes; and (*b*) the structure of the bands as well as the meaning of the longitudinal differentiation of staining bands and of the non-staining interbands (Painter and Griffen, 1937; Metz, 1941; White, 1954; Alfert, 1954; Ris, 1957). As to the cause of the increase in width there is considerable evidence (Slizynski, 1950; White, 1954; Ris, 1957) to show that these chromosomes are polytene (multistranded). As to the reason for the increase in length we still have no clear idea. Darlington (1955) and Ris (1957) explain the great length of these chromosomes as a result of the uncoiling of the original chromonemata, but as we will show later some recent data (see p. 326) indicate that during larval development there are differential increases of the contents of DNA, RNA, and protein at different loci of the chromosomes, varying in tissues as well as with the age of the larva, which could explain the increased length of these chromosomes. This would not eliminate the possibility of an uncoiling of the chromonemata which in this particular case was neither proved nor disproved.

Concerning the structure of these chromosomes, there is also no agreement among different authors who have worked on polytene chromosomes. The main reason for the disagreement is the lack of suitable methods and material to test the existing theories (Painter and Griffen, 1937; Metz, 1941; White, 1954; Alfert, 1954; Ris, 1957).

Changes Observed in the Bands of Polytene Chromosomes and the Problem of Chromosomal Differentiation

The so-called "bands" in polytene chromosomes are in reality "disks" which extend entirely across the chromosome transversally, like coins in a cylinder (Metz, 1941). They are very numerous in each chromosome, some being very thin, others thick, and still others of an intermediate width, well isolated or in close connection with other bands, each one however being recognized individually at least at some

stage of the animal's life. They are also very variable in their appearance during larval development. These variabilities of the bands during larval growth were known to earlier workers (Bridges, 1938; Poulson and Metz, 1938; Metz, 1941; Painter, 1942), but the type of changes, the time when they occur, as well as the probable meaning of the changes, were worked out in more detail only recently and independently by Beermann (1952, 1956), Mechelke (1953), Breuer and Pavan (1952, 1954, 1955), and Ficq and Pavan (1957) in Chironomidae and Sciaridae.

The main point in these studies was to follow the behavior of specific bands or chromosomal regions during larval development. As there are good reviews of the works of Beermann and Mechelke (Beermann, 1956, 1958), I shall give more emphasis to the experiments done with *Rhynchosciara* with which I am more familiar (Dreyfus *et al.*, 1951; Nonato and Pavan, 1951; Breuer and Pavan, 1952, 1954, 1955; Ficq and Pavan, 1957; Pavan, 1958).

Rhynchosciara is a rather large fly found in neotropical America, from Mexico to southern Argentina. In Brazil we recognize about a dozen species, two of which, *R. angelae* and *R. milleri*, we have described and worked with. *R. angelae*, the species with which we did more work, occurs in nature in groups of several hundred larvae, all of the same sex, age, and stage of development. Their development is synchronous, so that at any time each member of the group is doing exactly what the others are doing. This synchrony of development, associated with the excellent polytene chromosomes of various organs and the possibilities of analysing these chromosomes at any stage of larval life, makes *Rhynchosciara* an ideal organism in which to follow the development and metabolic changes in polytene chromosomes.

Taking a group of larvae and sampling it every day, one can follow the development of the group and in the end have a representation of the development of an individual larva. Repeating this in several groups of larvae, Breuer and Pavan (1952, 1955) could follow the behavior of specific regions of polytene chromosomes, in the salivary glands and in the Malpighian tubule cells.

The main changes observable in the bands of the polytene chromosomes are related to their condensation or dilutions (puffing) during larval development. Some of these changes are very small, others very evident as shown by Beermann (1952), Mechelke (1953), and Breuer and Pavan (1955). These changes normally start at a certain stage of larval development in a given organ and normally are reversible. As shown by Breuer and Pavan (1955) some of the changes are entirely reversible while others are not, since after the process is completed one can observe a concentration of DNA which is much greater than it was before the process of puffing.

Breuer and Pavan (1952) have shown also that during the puff formation in some regions of salivary chromosomes of *R. angelae* there is accumulation of basophilic Feulgen-negative material. This material is eliminated from the chromosome after the puffing and is released in the nuclear sap as micronucleoli. The RNAse digestion shows that one of the main chemical components of this Feulgen-negative material formed in the chromosome is RNA.

This RNA, as shown by Pavan and Breuer (1955), is formed not only in regions of the chromosomes where the puffs are evident, but along practically the entire length of the chromosomes, in a greater or a smaller quantity, depending upon the region of the chromosomes and the age of the larva.

Using autoradiographic methods and tracers, precursors of DNA (tritiated thymidine), Ficq and Pavan (1957) have shown that in *R. angelae*, during the puff

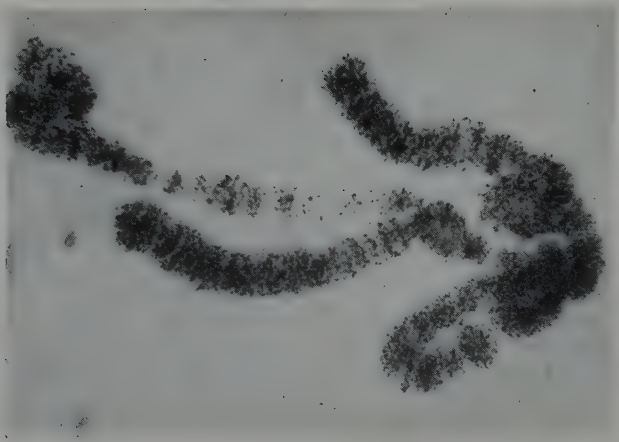


FIGURE 1. Photomicrograph of autoradiographed polytene chromosomes of a cell from the intestinal wall of a larva of *R. angelae*, previously injected with tritiated thymidine. The uniformity of incorporation in the euchromatin and heterochromatin suggests a duplication of the chromonemata (polytenization) at the time of the injection of the tracer.

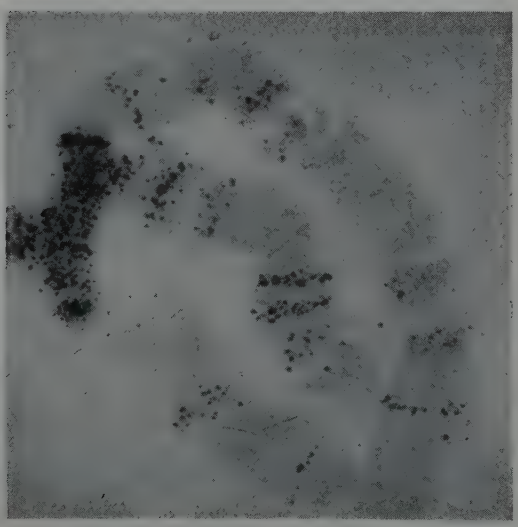


FIGURE 2. Photomicrograph of autoradiographed polytene chromosomes of a cell from the same animal and the same intestinal wall from which the cell of Figure 1 was prepared. Here the incorporation is shown only in special regions of the chromosomes, mainly in the heterochromatic chromocenter and in few of the euchromatic bands. This would be an increase of DNA (metabolic DNA?) independent of the process of polytenization of the chromosomes.

formation, the DNA plays a very important role. They also showed that during larval growth there is a series of duplications of the chromonemata (polytenization), and in addition to this uniform multiplication of the chromonemata, there are striking cases of differential increase of DNA at specific loci of the chromosomes at certain stages of larval life, which could occur simultaneously or independently of the polytenization of the entire chromosome (see Figs. 1, 2, and 3).

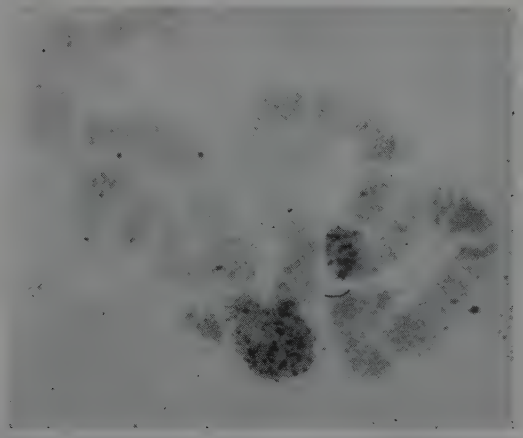


FIGURE 3. Photomicrograph of autoradiographed polytene chromosomes of a cell from the same animal and the same intestinal wall from which the chromosomes of Figure 1 and Figure 2 were prepared. In this figure the activity is shown only in the heterochromatic chromocenter without any polytenization or activities of euchromatic bands as shown in Figures 1 and 2.

The activity of DNA is more evident in chromosomal regions where the puffs are in process of development, and the intensity of incorporation of tritiated thymidine depends more on the stage of the larval life than on the quantity of DNA at a specific locus. Some bands, rich in DNA, may be very inactive at a certain stage of larval life, while others with much less DNA may be very active at that stage. Moreover, at another stage of larval development this inactive band may be very active, as shown by the incorporation of tritiated thymidine.

Pelc and Howard (1956), using a tracer technique in *Drosophila melanogaster,* found that there are regions of the salivary gland chromosomes of this species where the metabolism of RNA and proteins are independent of any activities of the DNA of these chromosomal regions. The inactivation of the DNA here is measured by the negative result obtained in the incorporation of the tracer (8-^{14}C adenine) by these chromosomal constituents, at bands which show an intense RNA metabolism.

Experiments are under way in *Rhynchosciara* to check the possibilities of RNA and protein metabolism being independent of the duplication or turnover, but as yet we do not have any positive results in this species (Ficq and Pavan, unpublished). The results at present show simultaneous activity of DNA and proteins in certain puffed regions of the chromosomes, as can be seen in Figure 4. The larva from which this figure was taken was injected simultaneously with tritiated thymidine and ^{14}C phenylalanine. With Ficq's method one can distinguish the two types

of particle ^{3}H in DNA and ^{14}C in protein. In some other experiments we have shown the simultaneity of metabolism of DNA and RNA in *R. angelae* (Ficq, Pavan, and Brachet, 1958).

The results of Pelc and Howard (1956) show that certain loci of the polytene chromosome of *D. melanogaster* may have an intense metabolism of RNA and protein not related to any activity of DNA. This situation in polytene chromosomes is similar to what probably happens in the lampbrush chromosomes, as we shall see later. Rudkin (1955), studying some puffs in the X- and the second chromosomes of *D. melanogaster,* found only increases of proteins without any changes in the nucleic acid content at a specific locus of the chromosome. Schurin (1957) showed that in *D. virilis* there are puffs in the salivary chromosomes where no increase of DNA or histone is observed, while the RNA and non-histone proteins are increasing. Stich and Nailor (1958a, b) presented spectographic evidence of this type of puff in Chironomidae, and besides this they showed, in the same organism, a puff where there is a differential increase of DNA which occurs also in puffs in *Rhynchosciara.* At the present X International Congress of Genetics a meeting was held of the people working on puffs in chromosomes, and the conclusion was reached that with the present data it is possible to distinguish various types of puffs in relation to the activities of the three main chemical components of the chromosomes: DNA, RNA, and proteins. It appears that, at least during certain stages of larval life, the increase in amount of each chemical component can be independent or may increase with one or both of the other two.

This being so, we have here a possibility for a longitudinal differentiation of the chromosome by an unequal increase in the proportions of its three main chemical components at different loci. This differential increase of the chemical component of the chromosomes is, in our opinion, the main reason for the great increase in length of the polytene chromosomes. Some of the substances produced are eliminated from the chromosomes while others are retained in it. This results in a longitudinal differentiation of the chromosome which is temporary for some components and permanent for others. It is interesting that the independence of metabolism between the RNA and the proteins, on the one hand, and the DNA, on the other, is known to occur in many organisms (Spiegelman, 1957).

Euchromatin and Heterochromatin

The differences in pattern, structure, and behavior of different parts of the chromosomes during cell division and metabolic stage permit a distinction between euchromatic and heterochromatic parts. Heitz (1928) introduced the term heterochromatin and was the first to make a study of the relative quantity and distribution of euchromatin and heterochromatin in mitotic and salivary chromosomes of *Drosophila* (Heitz, 1928, 1933, 1934; Pavan, 1946; Hannah, 1951).

Since then a great number of papers have been published on the distribution of these two parts of the chromosomes as well as on the function of each of them during cell division and metabolism, but the main points in these discussions are still under dispute (Schultz, 1947; Vanderlyn, 1949; Barigozzi, 1937; Hannah, 1951; Alfert, 1954). What looks clear today is that the differences between heterochromatin and euchromatin are more of degree than of kind, a view suggested by Poulson and Metz (1938) and accepted by many other authors (Hannah, 1951; Alfert, 1954; White, 1954).

The typical heterochromatin would be represented by the chromatic part of the chromosome near the centromere (or entire chromosomes, as the Y, super-

numerary, etc.), which shows a precocious condensation during early prophase compared with other parts of the chromosome which would be called euchromatic regions. In *Drosophila* the heterochromatic part of the salivary gland chromosomes contributes to the formation of the chromocenter at which the euchromatic arms are attached.

Less evident is the characterization of the so-called intercallary heterochromatin which was deduced by the results obtained in non-homologous association, or by increased breakability of chromosomes (Kaufmann, 1946; Schultz, 1947; Hannah, 1951; Alfert, 1954). It would not be distinguishable from euchromatin by cytological analyses or from the incidence of visible and lethal mutations, and would be distributed throughout the entire chromosome as single bands or long segments. This seems to us to be too specialized a distinction.

It has long been known that the heterochromatic parts of the chromosomes contain less mutable genes than corresponding euchromatic parts. The loss of heterochromatic sections was shown to have less effect than the corresponding deletion of euchromatic ones.

For these and other reasons, the heterochromatin was considered genetically less active than the euchromatin. On the other hand, heterochromatic regions have been shown to be genetically much more active than the euchromatin if one compares the position effects of these two types of chromatin on the neighboring genes (Demerec, 1940; Lewis, 1950; Hannah, 1951).

Commonly in the literature mention is made of cases of diverse concentrations of heterochromatin in different cells of the same organism (Demerec, 1940; Lewis, 1950; Hannah, 1951), but little attention was given to this.

Muller *et al.* (1937) showed that relatively long sections of the X-chromosome of *D. melanogaster* from the mitotic chromosomes are reduced to a single band in the salivary gland chromosomes. In the same species, Hinton (1942) showed that one-fifth of the left arm of mitotic II-chromosome is also reduced to a single band in the salivary chromosome. Pavan (1946) showed two types of heterochromatin in the chromosomes of *Drosophila nebulosa*, one of which shows a greater reduction than the other when mitotic and salivary chromosomes are compared.

It has long been known that the Y-chromosomes of many *Drosophila* species are reduced to a few chromomeres in the salivary chromosomes (Pavan, 1946; Schultz, 1947). Until very recently it was believed that the nucleic acid content of the nucleus was very variable during the mitotic cycle. The chromosomes would be very rich in nucleic acid during the metaphase and would lose it during the "resting" stage. The proof that DNA is an obligatory constituent of the chromosomes and that its amount is practically the same at the late "resting stage" and metaphase changes our chromosomal concept entirely (Boivin *et al.*, 1948; Mirsky and Ris, 1949; Swift, 1950).

As we have mentioned already, Boivin, Vendrely and Vendrely (1948), Mirsky and Ris (1949), Swift (1950) and others have found that the amount of DNA in diploid cells of various tissues of an animal is the same.

Lison and Pasteels (1951) and the other authors who believed in a possible variation in the amount of DNA had no direct proof as to how this variation could occur. One possible case of DNA variation not related to ploidy or elimination was given by Breuer and Pavan (1955) on polytene chromosomes of *Rhynchosciara angelae* analysed during larval development. Using the Feulgen technique, these authors could see a disproportional increase of DNA in certain bands of the salivary gland chromosomes at specific stages of the larval life (see Fig. 5).

This finding was subsequently confirmed by spectrographic method (Rudkin and Corlette, 1957) and by autoradiography (Ficq and Pavan, 1957). Figure 5 shows the type of chromosome C from the salivary gland of *R. angelae*, a, b, c, and d a stained chromosome, and a′ b′ c′ and d′ by autoradiography. What one can see by the Feulgen technique is repeatable with the incorporation of tritiated thymidine and respective autoradiograph. With the autoradiographic method, Ficq and Pavan (1957) showed that the incorporation of tritiated thymidine in the puffs studied represents duplication of DNA rather than a simple turnover or exchange of H atoms. Animals injected with the tritiated thymidine and analysed one to seven days after the injection show approximately the same amount of the tracer incorporated at specifi loci. With the data available in *Rhynchosciara*, we cannot for the moment say if there is or is not a turnover of DNA represented by the incorporation of tritiated thymidine.

Pavan and Sauaia (unpublished) made similar studies on the behavior of the heterochromatic portions of the polytene chromosomes of *R. angelae*. They found that here also there is a disproportional increase of the DNA during larval development. The difference here is that, while in the bands examined by Ficq and Pavan the great activity of DNA, shown by the high incorporation of tritiated thymidine, is present only once for each band at a specific age of the larva, the heterochromatic regions show active incorporation at many different stages of larval life. Unfortunately, we still do not have sufficient data to decide whether the incorporation of tritiated thymidine at various times is due to the same series of genes, which would be active and inactive several times, or to a series of different genes, each series reacting at a specific time, as do the euchromatic genes observed by Ficq and Pavan. The various types of heterochromatic bands near the centromere regions of the chromosomes, distinguishable at some stages of larval life, would be a good argument in favor of activities of different genes at different times, but the frequency with which the activity of heterochromatic regions occurs is an argument for repeatable activity of the heterochromatin at different stages.

The hypothesis of the activity of different heterochromatic bands at different times would explain the results obtained by Heitz (1928) and Pavan (1946) on the two types of heterochromatin in salivary gland nuclei of *D. virilis* and *D. nebulosa* respectively. The compact heterochromatin in salivary chromosomes of *D. virilis*, in the light of our present data, would be inactive at the time when the preparation was made while the diffuse type would be active. In *D. nebulosa* the two types of heterochromatin would be equally active at the mitotic prophase, while in the salivary chromosome one of them would be very active and present in a proportional quantity while the other which in these chromosomes would be practically inactive would be represented by only a few chromomeres. The same argument would be applied to the diverse concentration of heterochromatin in different tissues of *Drosophila melanogaster* found by Muller *et al.* (1937) and Hinton (1942), as well as to the different varieties of chromatin mentioned by White (1954).

In *R. angelae* the heterochromatin is more or less equally distributed between the four polytene chromosomes (Dreyfus, *et al.*, 1951; Breuer and Pavan, 1955) and our recent data show that in many instances there is a simultaneity and a uniformity in the incorporation of tritiated thymidine in heterochromatic regions of different chromosomes. These data would suggest that the different heterochromatic regions could have the same kind of genes.

In preparations of salivary chromosomes of old larvae of *R. angelae*, no common chromocenter is found. This lack could be an argument against the existence of

identical heterochromatic genes in different chromosomes. This, however, is not the case since in salivary chromosome preparations of young larvae and in polytene chromosomes from the intestines of larvae of any age, typical common chromocenters are observed as in *Drosophila* (see Figures 2 and 3). A similar situation is found in *Rhynchosciara milleri,* a species very close to *R. angelae*, but in which the existence of a chromocenter in preparations of young or old larvae is frequent even in chromosomes from the salivary gland (Pavan and Breuer, 1955).

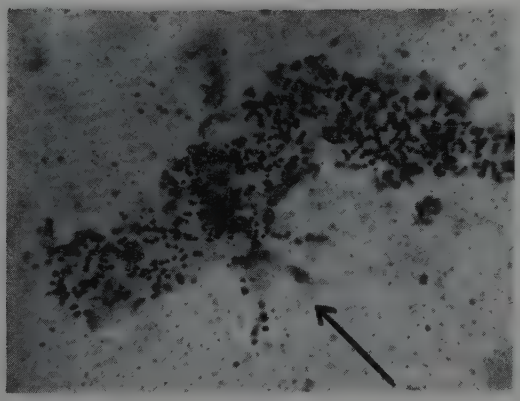


FIGURE 4. Photomicrograph of an autoradiographed distal end of chromosome C from a salivary gland cell nucleus. The larva was injected simultaneously with tritiated thymidine and ^{14}C phenylalanine. At the puffed region (arrow) one can see the two types of tracers, the soft ^{3}H and the hard ^{14}C, showing a disproportional increase of DNA and protein there.

One possible explanation of this behavior could be that duplication of identical heterochromatic genes from different chromosomes results in a common chromocenter while simultaneous duplications of different heterochromatic genes do not form it, and sometimes even destroy the common chromocenter previously formed.

The disproportional increase of DNA at the heterochromatic regions would be an argument in favor of the idea (Prokofieva and others) that heterochromatin corresponds to series of duplicated genes (Prokofieva-Belgovskaya, 1945; Pontecorvo, 1944; Schultz, 1947). This would in part explain the reason why the losses of heterochromatin are not as drastic in their effect as are losses of euchromatin. Males of *Drosophila melanogaster* may be formed without an entire Y-chromosome (they are morphologically normal but sterile) while in certain cases a zygote would die because of the absence of a single euchromatic band.

In wheat the formation of many nullisomics is possible, that is, plants in which a certain chromosome pair is wholly absent (Sear, 1944). Thus, in wheat the loss of euchromatic parts would have a less drastic effect than in *Drosophila*. Wheat being a polyploid, the absence of a pair of chromosomes does not mean that the corresponding genes are totally absent, but are only less numerous, since there are still other pairs of the same chromosome in the nucleus. In the loss of heterochromatic parts, in *Drosophila* for instance, a similar situation could occur since the loss of parts from one chromosome is compensated for by the presence of corresponding heterochromatic parts in other chromosomes.

The duplicate theory of heterochromatic genes would also explain why these parts of the choromosomes appear to have less mutable genes than does euchromatin. Any mutation in euchromatin in order to show itself has to overcome the effect of another gene when dominant, or wait to become homozygous when recessive. In the heterochromatin, on the other hand, a mutation would have to overcome the effect of a series of homologous genes, or wait for the very small probability of an individual being homozygous for that series of genes. This hypothesis would hold only if the heterochromatic gene were represented in a duplicated series of similar genes in the germ-line cells also, which possibility is by no means in disagreement with the cytological data available.

The Structure of the Puffed Region of the Chromosomes

From the hatching of the larva until the time when it pupates, very few mitotic divisions occur in its somatic cells. The growth is due mainly to an increase in the size of the cells rather than in numbers. As can be seen in Figure 6, the entire nucleus of the salivary gland cell from a young larva is no bigger than a single puffed band of the polytene chromosome of the same organ from an old larva.

The increases in width and length of polytene chromosomes during larval development are due to the multiplication of the chromonemata (polytenization) and to the differential increase of the permanent and transitory constituents of the chromonema. Enormous growth is observed in some regions of the polytene chromosomes during certain stages of larval life when specific bands go through a puffing process, increasing several times the diameter of the chromosome at the corresponding locus.

Poulson and Metz (1938), comparing different puffed regions of *Sciara*, concluded that these regions have structures which could be classified into two types, which they called "puffs" and "bulbs." The distinction of these two types is not clear to us from our experience in *Rhynchosciara,* a genus very close to *Sciara*. The puffed regions have no constant patterns since they are changeable structures, some of which change from a single band to an enormous puff, and back again to the banding stage. If we take a structure which would in Poulson and Metz's classification be called a typical puff, such as the one in the distal end of chromosome C from *R. angelae*, we see that some intermediate stage of this process could be called bulb in their sense.

On the other hand, it is possible that a real difference exists among the puffing regions of the polytene chromosomes. If interbands can puff, it would be possible to establish a difference between this type of chromosomal region swelling and the one resulting from the development of a band. As discussed previously there are puffing regions where the DNA presents a very high activity changing its concentration during the process, while in others DNA does not show any perceptible activity.

Beermann (1956) believes that diffuse swelling or puffing of the chromosomes could occur in the interbands. But in his work he does not give any clear example of an interband puff. Pavan and Breuer (1955) have shown that the concentration of RNA in polytene chromosomes is variable in space and time, in the puffed regions as well as the interbands along the entire length of the chromosome. They did not study in detail any of the puffs not directly related to the bands.

Detailed studies were done of "Balbiani ring" and puffs of Chironomidae and in puffs of *Rhynchosciara.*

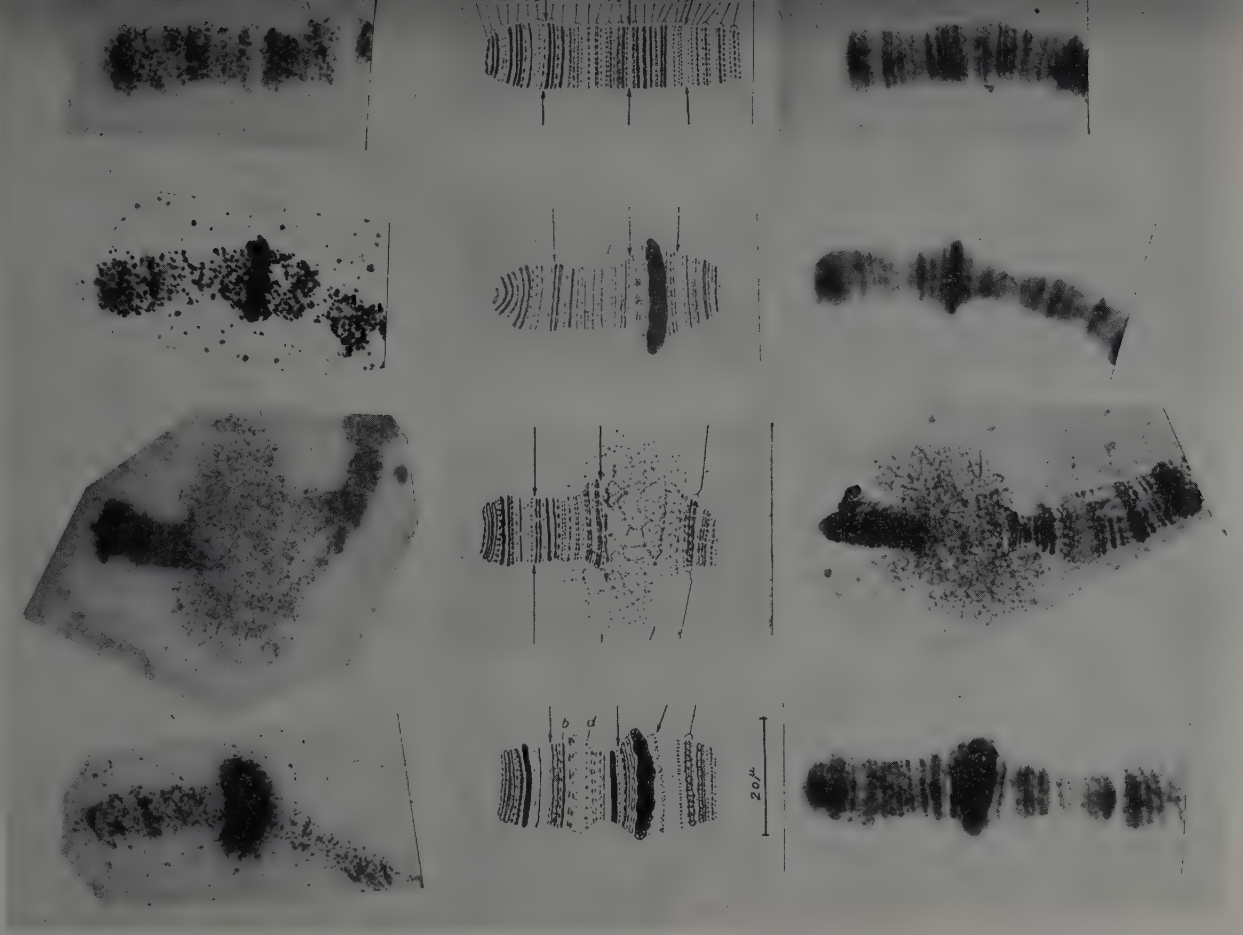


FIGURE 5. Distal end of chromosome C from the salivary gland of *R. angelae* at different stages of larval development. The left column shows picures of autoradiographic preparations of larvae injected with tritiated thymidine; the middle column shows *camera lucida* drawings of the chromosomes stained by orcein or by Feulgen; the column at right shows microphotographic plate of the chromosome prepared by acetic orcein. The horizontal row at the top shows corresponding chromosomal ends from full-grown larvae; the following horizontal rows show corresponding chromosomes from larvae at successive phases during the prepupation stage of the larva. For more detail of this puffing process see Breuer and Pavan (1955).

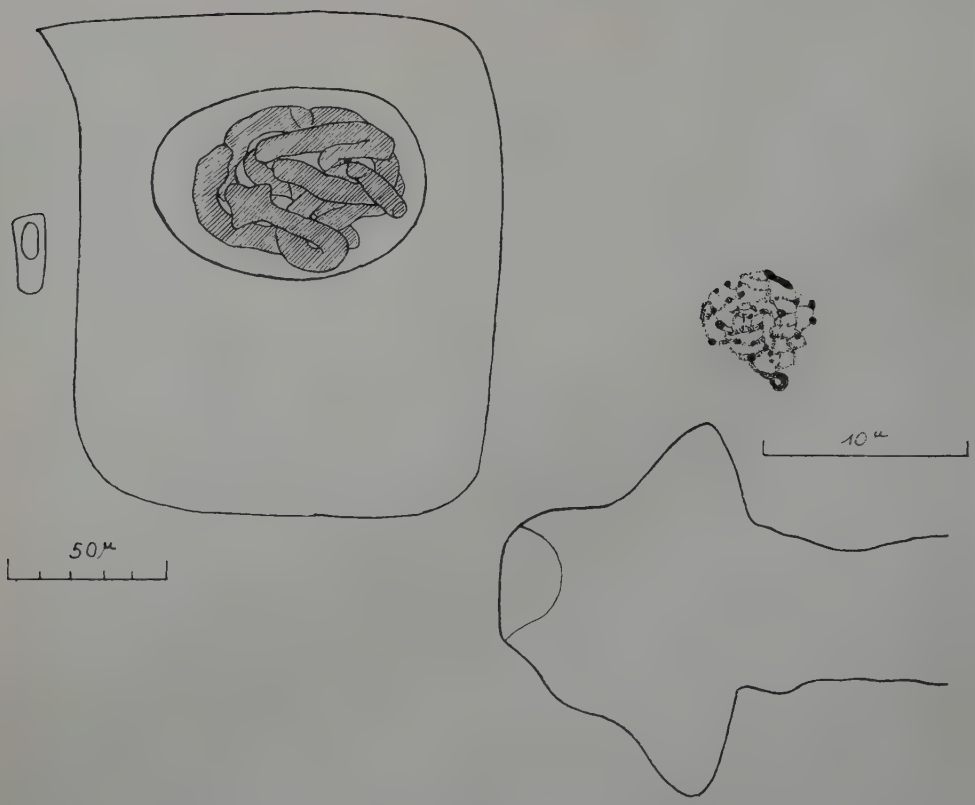


FIGURE 6. *Camera lucida* drawings of salivary gland cells and chromosomes of *R. angelae*. At the left we have drawings at the same magnification of corresponding salivary gland cells: the smaller one is from a larva one day old and the larger from a larva at the prepupal stage. One can see that the entire nucleus of a cell from a young larva has a volume no bigger than the volume of a puffed band of the polytene chromosome. This can be seen also in the drawing at the right, where an entire nucleus was drawn at the same magnification as the tip of chromosome B at the puffed stage.

Beermann (1956) believes that development of the Balbiani ring is due to the following factors: (1) a general torsional tension within the polytene cable; (2) local failure of the constituent fibrils, or "ultimate" chromonemata, to unite; (3) local mechanical weakening of the chromonemata. These factors together would cause the fibrils to bend out of the chromosome and to form loops. Thousands of such loops would compose the "Balbiani ring."

Breuer and Pavan (1952, 1954, 1955) see the development of the puffs in *Rhynchosciara* in another way. First, they have shown that, in the puffs which they studied in detail, there occurs a disproportional increase of DNA in the puffing region when compared with the neighboring ones. They have observed a very active interaction between the bands and the nuclear sap and, simultaneously with the increase in DNA, the formation of a Feulgen-negative substance inside of the band. During the formation of this new substance (mainly RNA) a progressive vacuolization begins inside the band which is divided into smaller and smaller vacuoles, until the puff is at its maximum and the DNA form an almost uniform net. All the space inside the net is filled by the newly formed basophilic, Feulgen-negative substance.

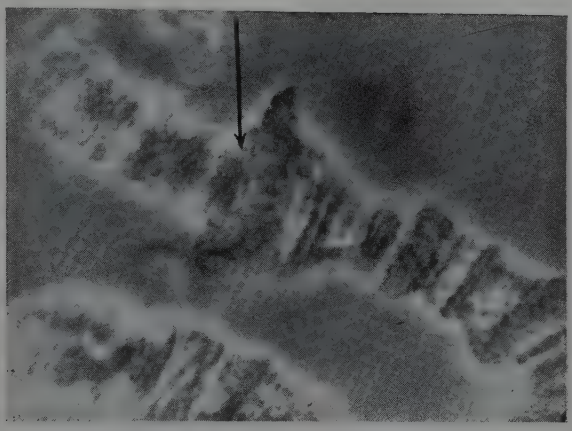


FIGURE 7. Microphotograph of the distal end of chromosome C from a salivary gland cell of *R. angelae* dissected in physiological solution and pictured in phase contrast microscope. Any pressure by the cover slip over the chromosome was avoided. The arrow indicates a region of thick bands which in this case do not permit the expansion of the puff in that direction by the invasion of the substances produced by the puffed band.

After this stage the process regresses, the newly formed Feulgen-negative substance is eliminated from the chromosome into the nuclear sap as a micronucleolus, and the chromosomal region returns to the banding stage. It is interesting to follow the behavior of bands near the one which goes through a puffing process. They act passively and while the diameter of the chromosome is increasing at the region of the puff, the neighboring bands are broken and the spaces left are invaded by the substances produced by the puffed band. The thinner bands break very easily, while the thicker ones may for some time resist and thus determine the shape of the puff (Fig. 7). In a later stage even the thick bands normally disintegrate into many blocks and the puff takes a symmetrical shape, having spread the newly produced substance to both sides, before returning to the banding stage.

The interpretation of Beermann of the structure of the "Balbiani ring" favors the idea of an analogy between the behavior and function of some parts of the salivary gland and those of lampbrush chromosomes. As shown by Ficq, Pavan, and Brachet (1958) however, this analogy cannot be generalized and would not hold, at least not if we consider the puffed regions studied in the polytene chromosomes of *Rhynchosciara*. In these latter puffs, during the metabolic process the DNA plays a very active role duplicating differentially in time and in space, while in the lampbrush chromosomes, if the DNA plays any role during the very high activity of the loops, it is not manifested by its duplication, or by the incorporation of tritiated thymidine as occurs in polytene chromosomes of *Rhynchosciara*.

DISCUSSION AND CONCLUSIONS

The observations of the polytene chromosomes from the same tissue at different ages of the larva permit the conclusion that genes have different activities at different stages of larval development. Analysis of the polytene chromosomes from different

tissues leads to the conclusion that the same gene acts differently in different tissues. The use of the spectrographic method, associated with the use of tracers, precursors of DNA, RNA and proteins, permits analysis of the activities of each one of these chemical components of the polytene chromosomes. With the available data it looks as if, at least at certain stages of the larval life, the activities of each one of these components may be independent or may run together with one or both of the other two. The observable data at present show the activities of these components at certain stages of the larval life, but we still do not have data on the interrelations of these components before they become very active, forming puffs or similar structures. It is possible to imagine that at the beginning, the formation of RNA or of the protein would depend on the DNA but later they would react independently.

On the other hand, we have very good evidence of disproportional increases of each of the three main chemical components of the chromosomes in the same tissue at different stages of larval life, as well as in different tissues at the same or different ages of the larvae. This disproportional increase of the chemical component would differentiate the present chromosome from the one from which it was derived. This would be a new possibility of chromosomal differentiation. Some of the substances formed in the chromosome during development would be eliminated from it subsequently, while others would be maintained in it, resulting in this way in a temporary and also in a permanent differentiation of the chromosome. In this respect it is interesting to keep in mind the possibilities of a similar differentiation in types of chromosomes other than the polytene. The results presented by Lima de Faria *et al.* (1958), at this Congress, on the structural differentiation of the chromosomes in different tissues of plants are pertinent to the subject.

The heterochromatin in polytene chromosomes of *Rhynchosciara* shows a very high activity at many different stages of the larval life. The maintenance of the tracers at the heterochromatic region for several days after the injection of the marked precursor suggest that in this part of the chromosome as it happens in some euchromatic bands we have a disproportional increase of DNA which would be an argument in favor of the theory of Prokofieva (1945) and others (Pontecorvo, 1944; Schultz, 1947) that the heterochromatin represents groups of duplicated similar genes.

Acknowledgments

I wish to express my appreciation to the Directors of the Rockefeller Foundation, Conselho Nacional de Pesquisas, and Comissão Nacional de Energia Nuclear for the grants which gave support to our investigations. The author is indebted to Mrs. Marta E. Breuer, Dr. A. Ficq, and Mr. Heni Sauaia for help during the course of the work and for the permission to use unpublished data. The author appreciates also the kindness of Professor Th. Dobzhansky and Dr. A. B. da Cunha in reading the manuscript and to Miss Therezinha Ungaretti for typing it.

REFERENCES

Alfert, M. 1954. Composition and structure of giant chromosomes. Internat. Rev. Cytol. *3*: 131–175.

Barigozzi, C. 1937. Primo contributo alla conoscenza di alcuni componenti dei cromosomi: Sostanze minerali e proteine nei cromosomi delle ghiandole salivari di chironomus. Zschr. Zellforsch. *26*: 462–472.

Beermann, W. 1950. Chromomerenkonstanz bei Chironomus. Naturwissenschaften *37*: 543–544.

——— 1952. Chromomerenkonstanz und spezifische Modifikationen der Chromosomenstruktur in der Entwicklung und Organdifferenzierung von *Chironomus tentans*. Chromosoma, Berl. *5*: 139–198.

——— 1956. Nuclear differentiation and functional morphology of chromosomes. Sympos. Quant. Biol. *21*: 217–232.

——— 1958. *In* Unesco Symposium at Edinburgh, 1957, ed. C. H. WADDINGTON. (in press).

BOIVIN, A., R. VENDRELY, et C. VENDRELY. 1948. L'acide d'ésoxyribonucléique du noyau cellulaire, dépositaire des caractères héréditaires: Arguments d'ordre analytique. C. rend. Acad. sc. *226*: 1061–1063.

BRACHET, J. 1957. Biochemical cytology. New York: Academic Press.

BREUER, M. E., e C. PAVAN. 1952. Gens na diferenciação. Cienc. e Cult., Brasil. *4*: 115.

——— 1954. Salivary chromosome and differentiation. Proc. IX Internat. Congr. Genet. Carylogia *6* (Suppl.): 778.

——— 1955. Behaviour of polytene chromosomes of rhynchosciara angelae at different stages of larval development. Chromosoma, Berl. *7*: 371–386.

BRIDGES, C. B. 1938. A revised map of the salivary gland x-chromosome of *Drosophila melanogaster*. J. Hered. *29*: 11–13.

COOPER, K. W. 1938. The nuclear cytology of the grass mite, pediculopsis granium (Reut.), with special reference to karyomerokinesis. Chromosoma, Berl. *1*: 51–103.

DARLINGTON, C. D. 1955. The chromosome as a physico-chemical entity. Nature, Lond. *176*: 1139–1144.

DEMEREC, M. 1940. Genetic behaviour of euchromatic segments inserted into heterochromatin. Genetics *25*: 618–627.

DREYFUS, A., E. NONATO, M. E. BREUER, and C. PAVAN. 1951. Polytene chromosomes in various organs of *Rhynchosciara angelae* Nonato and Pavan (Diptera). Rev. brasil biol. *11*: 439–450.

FICQ, A., and C. PAVAN. 1957. Autoradiography of polytene chromosomes of *Rhynchosciara angelae* at different stages of larval development. Nature, Lond. *180*: 983–984.

FICQ, A., C. PAVAN, and J. BRACHET. 1958. (in press).

HANNAH, A. 1951. Localization and function of heterochromatin in *Drosophila melanogaster*. Advance. Genet. *4*: 87–125.

HEITZ, E. 1928. Das Heterochromatin der Moose, I. Jahrb. wiss. Bot. *69*: 762–818.

——— 1933. Die somatische Heteropyknose bei *Drosophila melanogaster* und ihre genetische Bedeutung. (Cytologische Untersuchungen an Dipteren. III). Zschr. Zellforsch. *20*: 237–287.

——— 1934. Ueber A- und B-Heterochromatin sowie Konstanz und Bau der Chromomeren bei *Drosophila*. Biol. Zbl. *54*: 588–609.

HINTON, T. 1942. A comparative study of certain heterochromatic regions in the mitotic and salivary gland chromosomes of *Drosophila melanogaster*. Genetics *27*: 119–127.

KAUFMANN, B. P. 1946. Organization of the chromosome. 1. Break distribution and chromosome recombination in *Drosophila melanogaster*. J. Exp. Zool. *102*: 293–320.

LEWIS, E. B. 1950. The phenomenon of position effect. Advance. Genet. *3*: 73–115.

LISON, L., et J. PASTEELS. 1951. Etudes histophotométriques sur la teneur en acide désoxyribonucléique des noyaux au cours du développement embryonnaire chez l'Oursin *Paracentrotus lividus*. Arch. biol., Par. *62*: 1–43.

LISON, L. et V. VALERI. 1956. L'erreur dans la détermination histophotométrique de l'acide désoxyribonucléique et la variation individuelle des noyaux en acide désoxyribonucléique. Chromosoma, Berl. *7*: 497–507.

MAINX, F. 1949. The structure of the giant chromosomes in some Diptera. Proc. VIII Internat. Congr. Genet., Hereditas (Suppl.) pp. 622–623.

MAKINO, S. 1938. A morphological study of the nucleus in various kinds of somatic cells of *Drosophila virilis*. Cytologia *9*: 272–282.

MATHER, K. 1948. Nucleus and cytoplasm in differentiation. Sympos. Soc. Exp. Biol., Cambr. *2*: 196–216.

MECHELKE, F. 1953. Reversible Strukturmodifikationen der Speicheldrüsenchromosomen von *Acricotopus lucidus*. Chromosoma, Berl. *5*: 511–543.

——— 1958. The timetable of physiological activity of several loci in the salivary gland chromosomes of *Acricotopus lucidus*. Proc. X Internat. Congr. Genet. *2*: 185.

METZ, C. W. 1941. Structure of salivary gland chromosomes. Sympos. Quant. Biol. *9*: 23–39.

MIRSKY, A. E., and H. RIS. 1949. Variable and constant components of chromosomes. Nature, Lond. *163*: 666–667.

MOORE, B. C. 1952. Desoxyribose nucleic acid in embryonic diploid and haploid tissues. Chromosoma, Berl. *4*: 563–576.

MOORE, B. 1957. DNA in diploid and androgenetic amphibian hybrids. J. Morph. *101*: 227–274.

MULLER, H. J.; D. RAFFEL; S. M. GERSHENSON; and A. A. PROKOFIEVA-BELGOVSKAYA. 1937. A further analysis of loci in the so-called "inert region" of the x chromosome of *Drosophila*. Genetics *22*: 87–93.

NONATO, E., and C. PAVAN. 1951. A new species of "*Rhynchosciara*" Rubsaamen, 1894. Rev. brasil biol. *11*: 435–437.

PAINTER, T. S., and A. B. GRIFFEN. 1937. The structure and the development of the salivary gland chromosomes of simulium. Genetics *22*: 612–633.

PAINTER, T. S. and A. N. TAYLOR. 1942. Nucleic acid storage in the toad's egg. Proc. Nat. Acad. Sc. U.S. *28*: 311–317.

PASTEELS, J., et L. LISON. 1950. Teneur des noyaux au repos en acide désoxyribonucléique dans différents tissus chez le rat. C. rend. Acad. sc. *230*: 780–782.

PAVAN, C. 1946. Two types of heterochromatin in *Drosophila nebulosa*. Proc. Nat. Acad. Sc. U.S. *32*: 137–145.

——— 1958. *In* Unesco Symposium at Edinburgh, 1957, ed. C. H. WADDINGTON. (in press).

PAVAN, C., and M. E. BREUER. 1952. Polytene chromosomes in different tissues of *Rhynchosciara*. J. Hered. *43*: 151–157.

——— 1955. Polytene chromosomes of *Rhynchosciara milleri* sp. n. (Diptera, mycetophilidae). Rev. brasil biol. *15*: 329–339.

——— 1955. *In* Symposium on Cell Secretion, ed. G. SCHREIBER, p. 90. Belo Horizonte, Brazil: Universidade de Minas Geraes.

PELC, S. R., and A. HOWARD. 1956. Metabolic activity of salivary gland chromosomes in diptera. Exp. Cell Res. *10*: 549–552.

PONTECORVO, G. 1944. Structure of heterochromatin. Nature, Lond. *153*: 365–367.

POULSON, D. F. and C. W. METZ. 1938. Studies on the structure of nucleolus-forming regions and related structures in the giant salivary gland chromosomes of Diptera. J. Morph. *63*: 363–382.

PROKOFIEVA-BELGOVSKAYA, A. A. 1945. Heterochromatization as a change of chromosome cycle. J. Genet., Cambr. *48*: 80–98.

RIS, H. 1957. Chromosome structure. *In* A Symposium on the Chemical Basis of Heredity, eds. W. D. MCELROY and B. GLASS, pp. 23–69. Baltimore: The Johns Hopkins Press.

RUDKIN, G. T. 1955. The ultraviolet absorption of puffed and unpuffed homologous regions in the salivary gland chromosomes of *Drosophila melanogaster*. Genetics *40*: 593 (Abstract).

RUDKIN, G. T., and S. L. CORLETTE. 1957. Disproportionate synthesis of DNA in a polytene chromosome region. Proc. Nat. Acad. Sc. U.S. *43*: 964–968.

SCHULTZ, J. 1947. The nature of heterochromatin. Sympos. Quant. Biol. *12*: 179–191.

SCHURIN, M. F. 1957. Studies on the cytochemistry and fine structure of *Drosophila virilis* salivary gland nuclei. Thesis, Univ. of Chicago.

SEARS, E. R. 1944. Cytogenetic studies with polyploid species of wheat. II. Additional chromosomal aberrations in *Triticum vulgare*. Genetics *29*: 232–246.

SLIZYNSKI, B. M. 1950. *Chironomus* versus *Drosophila*. J. Genet., Cambr. *50*: 77–78.

SPIEGELMAN, S. 1957. Nucleic acids and the synthesis of proteins. *In* A Symposium on the Chemical Basis of Heredity, eds. W. D. MCELROY and B. GLASS, pp. 232–267. Baltimore: The Johns Hopkins Press.

STICH, H. F., and J. M. NAYLOR. 1958a. Variation of desoxyribonucleic acid content of specific chromosome regions. Exp. Cell Res. *14*: 442–445.

——— 1958b. Variation of the DNA, RNA and protein content of specific chromosome regions during development. Proc. X Internat. Congr. Genet. *2*: 276.

SWIFT, H. F. 1950. The desoxyribose nucleic acid content of animal nuclei. Physiol. Zool. *23*: 169–198.

TRAGER, W. 1935. The relation of cell size to growth in insect larvae. J. Exp. Zool. *71*: 489–508.

VANDERLYN, L. 1949. The heterochromatin problem in cytogenetics as related to other branches of investigation. Bot. Rev. *15*: 507–582.

WHITE, M. J. D. 1954. Animal Cytology and Evolution. Cambridge: Cambridge Univ. Press.

WRIGHT, S. 1941. The physiology of the gene. Physiol. Rev. *21*: 487–527.

CONTRIBUTION TO THE PHYSIOLOGICAL AND BIOCHEMICAL GENETICS OF PTERIDINES AND PIGMENTS IN INSECTS

Ernst Hadorn[1]

The use of paper chromatographic methods has shown that different genotypes of *Drosophila melanogaster* are characterized by the various fluorescent substances they contain (Hadorn and Mitchell, 1951; Hadorn, 1954a). Similar results were obtained from studies on the meal moth, *Ephestia kühniella* (Hadorn and Kühn, 1953) and on the silkworm, *Bombyx mori* (Nawa and Taira, 1954; Sakaguchi and Tsujita, 1955). Most of these gene-conditioned substances were shown to be different pteridine compounds (Forrest and Mitchell, 1954a, b, 1955; Viscontini *et al.* 1955a, b; De Lerma and De Vincentiis, 1955). It is the aim of this report to review a few of the recent findings concerning pteridine metabolism. In doing so we shall concentrate mainly on the experiments carried out with *Drosophila*. Though a steadily increasing store of promising data is now available, no final conclusions as to the biosynthetic and physiological relations of the different pteridines are possible at the present time.

Inventory of Pteridines

Three red eye pigments: Drosopterin, isodrosopterin, and neodrosopterin (Viscontini, Karrer, and Hadorn, 1957). These closely related compounds differ mainly in their optical activity. One of them, isodrosopterin, has been isolated in crystalline form (Viscontini, 1958a). Although in *Drosophila melanogaster* drosopterins are restricted to the imaginal eyes, in *Drosophila pseudobscura*, *D. subobscura*, and *D. obscura* they occur in testes as well (Graf and Hadorn, 1959). Drosopterins have so far not been reported in insects outside the family Drosophilidae.

Isoxanthopterin. This deep violet-blue fluorescent compound is found in all groups of arthropods tested so far (cf. Hadorn and Schwinck, 1956). Among a great number of *D. melanogaster* genotypes investigated, only the mutants *rosy* (*ry*) and *maroon-like* (*ma-l*) lack isoxanthopterin. *Drosophila* males contain several times more isoxanthopterin than females. This sex difference is due mainly to an accumulation in the testes. But other organs too, for instance the eyes, are richer in isoxanthopterin in the male than in the female (see p. 351).

2-Amino-4-hydroxypteridine (= HB_1 of Viscontini *et al.*, 1955a, b) is the direct precursor of isoxanthopterin (see p. 343). Accordingly, we find this pteridine, which emits a fluorescence sky-blue in color, in all genotypes where isoxanthopterin is found. In *rosy* the HB_1 (himmelblau) appears in much increased quantity (see p. 344).

Biopterin (*2-amino-4-hydroxy-6-(1′, 2′-dihydroxypropyl) pteridine*) (= HB_2 of Viscontini *et al.*, 1955; Forrest and Mitchell, 1955) is a highly light-sensitive pteridine of general occurrence in *Drosophila* and *Ephestia*.

[1]Zoologisch-vergl. anatomisches Institut der Universität Zürich, Zürich, Switzerland. In preparing this report I enjoyed the assistance of Dr. Elisabeth Goldschmidt and Dr. G. E. Graf. Our experiments were supported by grants from the Georges and Antoine Claraz-Schenkung.

Sepiapteridine is a yellow pigment with a strong yellow fluorescence. It is highly concentrated in the eyes of the *sepia* (*se*) mutant of *Drosophila melanogaster* and seems to be identical with the xanthopterin-B found in the silkworm (Nawa and Taira, 1955). It was first isolated from *sepia* flies by Forrest and Mitchell (1954a).

Xanthopterin (greenish blue fluorescence) is a characteristic constituent of *Ephestia* (Viscontini, Kühn, and Egelhaaf, 1956). In *Drosophila* it appears in chromatograms after development with basic solvents (Ziegler-Günder and Hadorn, 1958).

2-Amino-4-hydroxypteridine-6-carboxylic acid. Here we are dealing with a product which appears in chromatograms not sufficiently protected from light. It is formed by degradation of drosopterins, sepiapterin, and biopterin. However, small amounts might occur naturally.

There are a few more pteridine compounds present in insects. But since they are not yet identified chemically and because they cannot play a role in the following discussion, we shall not take them into consideration. We should, however, mention the general occurrence of riboflavin in insects. It is very difficult to distinguish it from the sepiapteridine with which it shares the fluorescent yellow color and the Rf-value in most of the current solvents.

Patterns of Biochemical Pleiotropism

According to previous reports (Hadorn and Mitchell, 1951; Hadorn and Kühn, 1953; Hadorn, 1954a, 1956a) mutations which affect the visible eye colors of *Drosophila* and *Ephestia* manifest themselves locus-specifically in the inventory of pteridines. When the wild type is used as a basis for comparison, it is seen that some pteridines fail to appear in certain mutants, others are present in reduced amounts, and still others are found in greatly increased quantities. Figure 1 illustrates this phenomenon for a few genotypes of *Drosophila melanogaster*. The drosopterins (DP) are reduced in *rosy* (ry^2) and fail to appear in *lozenge-clawless* (lz^{cl}) and *sepia* (*se*). Isoxanthopterin (IX) is hardly affected by lz^{cl} but is lacking in ry^2. There is less xanthopterin (X) in ry^2 and lz^{cl} than in wild type, whereas the *sepia* mutant exhibits a considerable increase of this compound. The 2-amino-4-hydroxypteridine (HB_1) accumulates highly in ry^2 and *se* and is much diminished in lz^{cl}. Whole imagoes of ry^2 and lz^{cl} contain more biopterin (HB_2) than wild-type controls; the same substance becomes still more increased in *se*. The amount of sepiapteridine (SP) is much exaggerated in the *se* mutant.

None of the mutants described so far possessed a new pteridine which would be alien to wild type.

From data such as those presented in Figure 1 it is hardly feasible to draw conclusions concerning the biochemical and physiological interrelations between different compounds. Each mutation influences the pattern in its own fashion. In one genotype the decrease or loss of one substance is accompanied by an increase of another pteridine, whereas in another genotype the two compounds are positively correlated, and in a third mutant only one of the pteridines is affected (Hadorn, 1956a). For this reason results based on only one or a few mutants can lead to a faulty interpretation. Statements concerning the sequence of genic interactions which lead to the biochemical pleiotropism should rather be based on direct experimental evidence.

Moreover, the biochemical and physiological genetics of pteridine metabolism is complicated because mutations seem to affect different organs in a desultory way.

Thus, it could be shown that the abdomen of the *biochemica* mutant (*bch*) of *Ephestia* contains all pteridines characteristic of wild type, while in *bch* eyes only 2-amino-4-hydroxypteridine and isoxanthopterin can be formed or deposited (Hadorn and Egelhaaf, 1956; Hadorn, 1956a; Viscontini, Kühn, and Egelhaaf, 1956). Such organ-specific modifications of pleiotropic patterns are found in all or most of

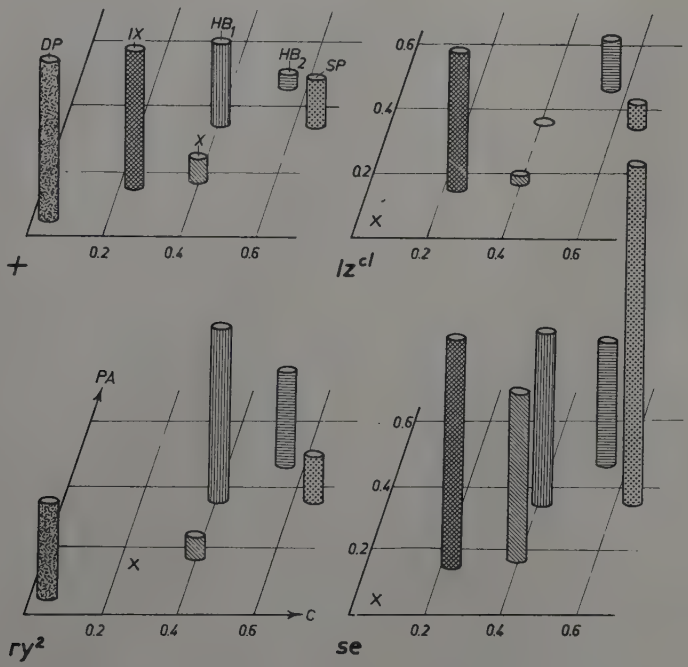


FIGURE 1. Pteridines in males of *Drosophila melanogaster*, aged three days. The heights of the columns represent the relative quantities of pteridines in two-dimensional chromatograms (Rf values in propanol-ammonia = PA and in collidine = C. Genotypes: +, wild type; lz^{cl}, *lozenge-clawless*; ry^2, *rosy*^{2}; *se*, *sepia*. Substances: DP, drosopterins; IX, isoxanthopterin; X, xanthopterin; SP, sepiapteridine; HB_1, 2-amino-4-hydroxypteridine; HB_2, biopterin. A cross indicates the lack of the respective substance.

the mutants in which pteridine metabolism is affected. We shall discuss only one more example. As seen in Figure 1 the mutant ry^2 of *Drosophila* contains much more 2-amino-4-hydroxypteridine (HB_1) than wild type. Figure 2 shows that this difference is manifest in all organs tested so far, though a large part of the enrichment in HB_1 is due to an accumulation in the testes. With regard to biopterin (HB_2, Figure 2) the different cellular systems react to the ry^2 mutation in strangely different directions. An increase in the eyes is paralleled by a sharp decrease in the malpighian tubes and in the testes. Since both HB_1 and HB_2 are clearly affected by the *ry* locus, the formation of these substances should be connected somewhere, somehow. But conflicting interpretations suggest themselves according to whether we judge from the quantities found in whole flies, in eyes, or in testes and malpighian tubes.

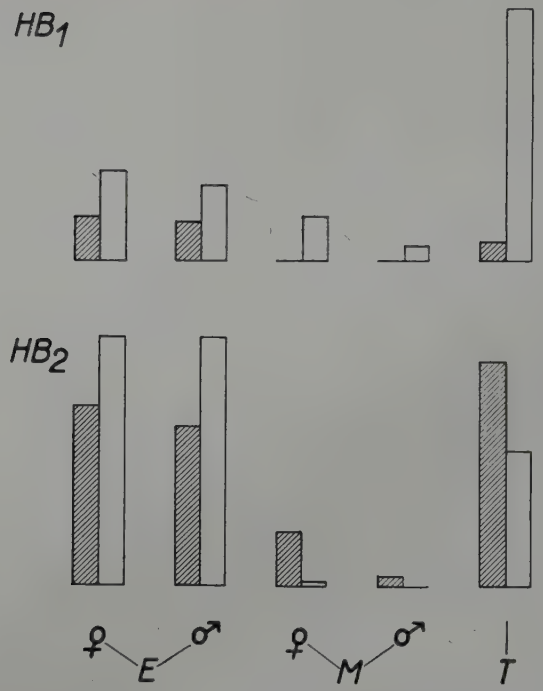


FIGURE 2. Relative amounts of 2-amino-4-hydroxypteridine (HB_1) and biopterin (HB_2) in eyes (E), malpighian tubes (M) and testes (T) of four-day-old imagoes of *Drosophila melanogaster*. Wild type (hatched columns) compared with *rosy* (white columns). After G. Handschin (unpublished).

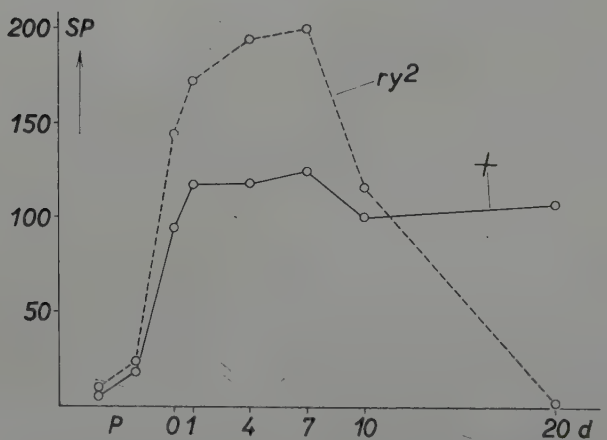


FIGURE 3. Developmental changes in the quantities of yellow fluorescent pigment (mainly sepiapteridine). Mean values in eyes of + and ry^2 males (*Drosophila melanogaster*) in pupae (P) and adults (0–20 days after hatching). From unpublished data of G. Handschin.

The evaluation of biochemical pleiotropism must, however, not only take into account the patterning by organ-specific competences but must also consider unexpected changes along the time axis of development. Some years ago we showed that the lack of pteridines in *white* and *brown* adults of *Drosophila melanogaster* is not due to an inability of these genotypes to synthesize pteridine (Hadorn, 1954b, 1956a). With the exception of drosopterins, probably all pteridines of wild type appear during metamorphosis although in more or less reduced amounts. But these metabolites disappear shortly after the hatching of the *w* and *bw* flies. We find the metabolites in high concentrations in the excretory material released with the meconium (Hadorn and Kürsteiner, 1956). Another example of a developmental change is illustrated in Figure 3. In eyes of the ry^2 mutant the quantity of yellow fluorescent pigment (which is most probably constituted mainly by sepiapteridine, but might also contain some riboflavin) rises shortly after pupation until a maximum is reached around the seventh day after hatching. Later on this compound gradually disappears from the ry^2 eyes. In twenty-day-old imagoes measurable amounts are no longer found. In wild type (+) the same fluorescent fraction never reaches the high level which is characteristic of the young *rosy* flies, but in contrast to ry^2 the early imaginal level is maintained throughout life. Speculations as to the effect of the *ry* locus might lead to controversial conclusions depending upon whether conclusions are based on data gained from young or from old adults.

We have now seen how the pleiotropic patterns of genic action on pteridines are influenced by each mutation in its locus-specific way. Such patterns are further modified within each genotype by organ-specific properties. Finally, the inventory of gene-conditioned substances changes during development in whole individuals as well as in different organs of different genotypes in an unpredictable manner. We have, therefore, to face a very complex system which is determined by three parameters: (1) the unknown number of interacting genic loci; (2) the variety due to differences in reacting cellular systems; and (3) the developmental changes. Moreover, this system represents three latent pitfalls for any interpreter. We have, therefore, tried to hint how faulty conclusions could result from observations based on single mutations only and from disregarding the organ-specific patterning or the ontogenetic changes. Though we are aware of these dangers and, therefore, intend in the following discussion to refrain from generalizing, even the few conclusions which appear justified today may in the near future need revision.

Manifestation of Recessive Genes in Pteridine Metabolism

Eye-color mutants of *Drosophila melanogaster* such as *sepia* (*se*) and *white* (*w*) are conventionally classified as recessives because no visible characters (phenes) appear in heterozygotes which would allow one to distinguish a $+/se$ or $+/w$ genotype from a $+/+$ individual. By using chromatographic methods and by measuring the quantities of fluorescent substances, it could, however, be shown that at least in some pteridines the *se* as well as the *w* gene manifests itself in heterozygotes (Ziegler-Günder and Hadorn, 1958). From Figure 4 we see that the xanthopterin fraction in $se/+$ heterozygotes reaches a concentration clearly intermediate between the low standard of $+/+$ and the much increased level of se/se. Another significant manifestation is apparent in the HB fraction. Here too the *se* gene causes in $+/se$ an increase in the direction of se/se. With respect to sepiapteridine and drosopterin, on the other hand, *se* behaves as a recessive gene.

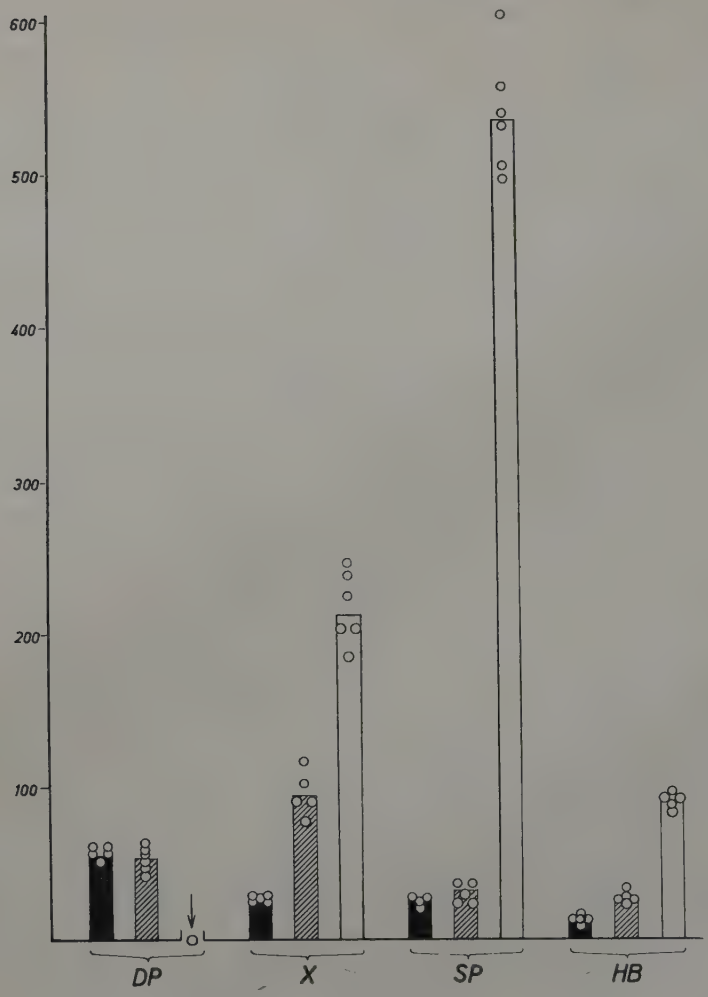


FIGURE 4. Histogram comparing units of fluorescence of different pteridine fractions in heads of +/+ (black), +/*se* (hatched) and *se*/*se* (white) male adults (5 days old). Individual measurements are indicated by small circles. DP, drosopterin; X, xanthopterin; SP, sepiapteridine; HB, HB_1, and HB_2. From Ziegler-Günder and Hadorn (1958).

Still more pronounced is the manifestation of *white* in heterozygotes (Figure 5). Here all pteridine fractions are affected in the eyes. The presence of the single *white* allele lowers the content in drosopterins and xanthopterin but causes a spectacular increase in the sepiapteridine and the HB fraction. By investigating different genotypes, it could be shown that we are dealing here with a genuine effect of the respective loci and not just with quantitative influences due to non-specific heterosis.

It is not possible to estimate at present, how general a phenomenon such a biochemical manifestation of recessives might be. But my collaborator G. E. Graf

(unpublished) found several other eye-color genes, such as *brown* (*bw*) and *sepiaoid* (*sed*), exerting a quantitative influence when present in heterozygotes. At all events, it is clear that the inventory of pteridines has proved to be most suitable for discovering hidden biochemical phenes of so-called recessive genes. What is found true for pteridines could be valid also for other metabolites. Some of these biochemical phenes might have selective significance and thereby contribute to balanced polymorphism or homeostasis.

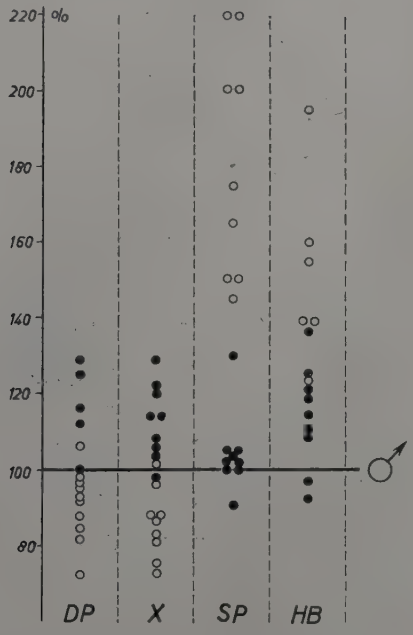


FIGURE 5. Relative amounts of pteridines in heads of +/+ females (black) and +/*w* sisters (white) of *Drosophila melanogaster*. The values are indicated in percentages of the quantities found in +/ brothers (100 per cent line). Abbreviations for pteridine fractions as in Figure 4. From Ziegler-Günder and Hadorn (1958).

ISOXANTHOPTERIN AND ITS PRECURSOR

The discovery that the mutant *rosy*^{2} of *Drosophila melanogaster* is unable to form isoxanthopterin (Hadorn and Schwinck, 1956) turned out to be valuable for more detailed studies of pteridine metabolism. It is now clear that this gene-conditioned block is due to the absence of xanthine dehydrogenase, an enzyme which oxidizes 2-amino-4-hydroxypteridine (HB_1) to isoxanthopterin (Forrest, Glassman, and Mitchell, 1956; Glassman and Mitchell, 1959).

Though it is known that isoxanthopterin is formed *in vitro* from HB_1 in the presence of milk xanthine oxidase (Forrest, Glassman, and Mitchell, 1956), it still remains to be proved that the isoxanthopterin of *Drosophila* also originates *in vivo*

from the HB_1 precursor. We have first of all the indirect evidence furnished by the fact that in the ry^2 mutant high quantities of HB_1 become accumulated. This is shown in Figure 1 for whole animals and in Figure 2 for single organs. It is probably no coincidence that the difference in the HB_1 content between + and ry^2 is most pronounced in the testes (Figure 2, T), that is, in those organs which in normal genotypes are highest in isoxanthopterin.

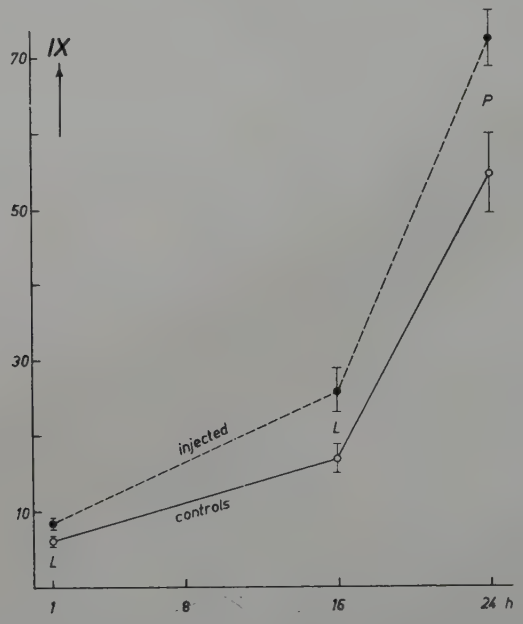


FIGURE 6. Quantitative differences in isoxanthopterin (IX) between + controls and individuals injected with 2-amino-4-hydroxypteridine (HB_1) during the third instar. Measurements are taken at 16 and 24 hours (h) after injection. The means and their standard errors are fluorescence values obtained from 4–10 individuals. After Graf, Hadorn, and Ursprung (1959).

More direct evidence is derived from experiments in which wild-type larvae were injected with synthetic HB_1 (Graf, Hadorn, and Ursprung, 1959). The organism provided with a surplus amount of the precursor reacts with an increase of isoxanthopterin (Figure 6). The utilization of HB_1 for the formation of isoxanthopterin is demonstrated, moreover, by implantation experiments. It is possible to induce the non-autonomous appearance of isoxanthopterin in *rosy* organs by implanting malpighian tubes or fat bodies from + donors (Hadorn and Schwinck, 1956; Hadorn, 1956a). If this additional substance is actually formed at the expense of the HB_1 precursor, we should find the two pteridines negatively correlated. Indeed, Figure 7 shows that the more isoxanthopterin a ry^2 host forms, the less HB_1 it contains (Hadorn and Graf, 1958). Analogous results were obtained in feeding experiments by Forrest, Glassman, and Mitchell (1956).

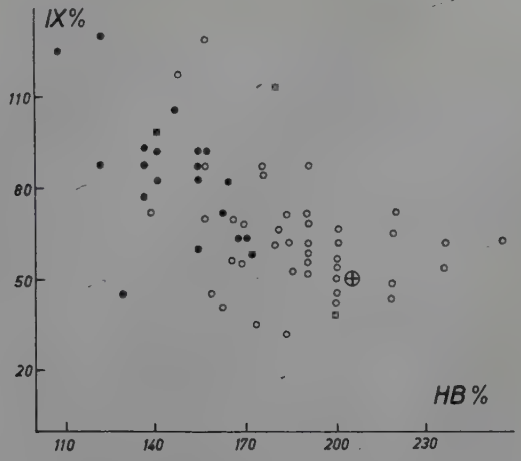


FIGURE 7. Correlations between 2-amino-4-hydroxypteridine (HB_1) and isoxanthopterin (IX) in single heads of *rosy*^{2} flies, aged four days, in which one (○) or two (●) pairs of + malpighian tubes have been implanted. The quantities are recorded as percentages of + controls (stock Sevelen). The encircled cross indicates the mean value for uninjected *ry*^{2} controls. If only isoxanthopterin had been measured the mean of *ry*^{2} controls should be at zero level. The value of 50 per cent is due to xanthopterin which overlaps the Rf position of isoxanthopterin after development with basic solvents. The increase in the *ry*^{2} hosts is however due to the addition of isoxanthopterin.

MUTANTS DEFICIENT IN XANTHINE DEHYDROGENASE

This enzyme has been isolated in purified form from wild-type *Drosophila melanogaster* by Glassman and Mitchell (1959). The preparation was active *in vitro* in oxidizing 2-amino-4-hydroxypteridine to isoxanthopterin. As expected from the lack of isoxanthopterin in *ry*^{2} no xanthine dehydrogenase (or xanthine oxidase) was found in this mutant. One is, therefore, justified in considering xanthine dehydrogenase as a gene-conditioned enzyme, the production of which depends *inter alias* on the *ry*$^+$ locus. Forrest, Glassman, and Mitchell (1956) found, moreover, that another mutant of *D. melanogaster*, *maroon-like* (*ma-l*) is deficient in xanthine dehydrogenase and consequently in isoxanthopterin. Apparently *ma-l* causes biochemical phenes which are most similar to *ry*^{2} and it turned out to be non-autonomous in gynandromorphic mosaics (Glassman, 1957), corresponding in this respect to the behavior of *ry*^{2} in transplantation experiments (Hadorn and Schwinck, 1956).

However, *ry*^{2} and *ma-l* differ in their immunological reactions to antibodies induced in rabbits by injecting wild-type xanthine dehydrogenase and, further, *ma-l* but not *rosy* exhibits a maternal effect (Glassman and Mitchell, 1959). Perhaps both loci, *ry*$^+$ as well as *ma-l*$^+$, might participate in the formation of the one

enzyme, xanthine dehydrogenase, possibly by the contribution of different constituents or precursors.

The lack of xanthine dehydrogenase in ry^2 explains a further biochemical property of this genotype. It is well known that this enzyme catalyzes the oxidation of hypoxanthine to xanthine, hence to uric acid. Accordingly, we found no uric acid in ry^2 but instead hypoxanthine as an excretion product (cf. Hadorn, 1956b; Mitchell, Glassman, and Hadorn, 1959).

The fact that a one-locus mutation affects purine as well as pteridine metabolism might be of general significance by demonstrating a most interesting mechanism leading to pleiotropy. Phenes of pleiotropic patterns are often found to be related in such a way that more secondary phenes are subordinated to primary ones which are nearer to the initial genic action. But this hierarchic relationship need not be the only one possible.

Relations between Drosopterins and Other Pteridines

In ry^2 only a reduced amount of red eye pigments (drosopterins) is formed. By exposing eye primordia of ry^2 to the influence of + organs, the mutant eye is enabled to reach a normal drosopterin content (Hadorn and Schwinck, 1956; Hadorn, 1956a). It was further found that the additional quantity of drosopterin formed by a + implant-bearing ry^2 host is positively correlated to the induced amount of isoxanthopterin (Hadorn and Graf, 1958). This relation is shown in Figure 8. All these facts could suggest an interpretation according to which the lack of xanthine dehydrogenase in ry^2 would also be responsible for the deficit in drosopterins.

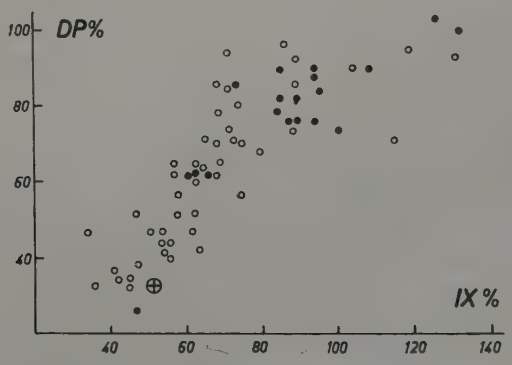


Figure 8. Correlations between isoxanthopterin (IX) and drosopterins (DP) in heads (eyes) of $rosy^2$ flies to which one (○) or two (●) pairs of + malpighian tubes have been implanted. Symbols as in Figure 7. From Hadorn and Graf (1958).

Such a simple explanation must be confronted, however, with the following observations. Numerous eye-color mutants of *Drosophila* have been investigated in transplantation experiments (Beadle and Ephrussi, 1936; Hadorn and Schwinck, 1956). With the exception of ry^2 and *ma-l* all of a large series tested behaved autonomously with regard to their deficit in drosopterins. Since, on the other hand,

such mutants form isoxanthopterin and uric acid, they must contain xanthine dehydrogenase (see also Taira, Nawa, and Oshima, 1957). This was once more directly shown by using drosopterin-deficient mutants such as *w*, *bw*, *se*, w^a, *ca*, *ma* and *p* as hosts for ry^2 eyes when they proved to be as potent as wild types in normalizing the pteridines of the implant (Hadorn and Schwinck, 1956). The low level in drosopterins in ry^2 could nevertheless be a consequence of the enzymatic defect. In this case, it is true, we had to assume that mutants such as *white* and *brown* are deficient in red eye pigments because some other prerequisites are affected.

Hypotheses regarding the metabolic relations of the drosopterins to other pteridines must remain speculative as long as the chemical constitution of the red eye pigments is not definitely known. Although most recent investigations have shown the drosopterins to be closely related in structure to the other pteridines occurring in *Drosophila* (Viscontini, 1958b), their immediate precursors are still unknown. It is true that various observations have led several authors to suggest that sepiapteridine, which accumulates in the *se* mutant, is metabolized to red eye pigments in the se^+ genotypes (Forrest and Mitchell, 1954; Danneel, 1955; Nawa and Taira, 1955; De Vincentiis, 1956). Experiments planned to test this hypothesis yielded, however, somewhat controversial results. In ry^2 eyes which developed as implants in + hosts, we found the induced increase of drosopterins accompanied by a clear decrease in sepiapteridine. But no such correlation is apparent in eyes of ry^2 hosts into which + malpighian tubes were implanted. Though here too the drosopterin content became normalized, no significant decrease in sepiapteridine was observed (Hadorn and Graf, 1958).

We attempted, moreover, to promote drosopterin production in ry^2 eyes by injecting different pteridines, such as HB_1 and isoxanthopterin which suggest themselves as possible precursors. None of these substances influenced the red eye pigments of the recipient (Graf, Hadorn, and Ursprung, 1959). In conclusion we feel that no definite statement as to the metabolic steps and the substances involved in drosopterin synthesis is possible as yet.

Such a cautious attitude is the more indicated since in different experimental set-ups the drosopterins may be correlated with certain other pteridines either positively or negatively. It follows from Figure 9a that a decrease in temperature from about 28°C. to 18°C. leads in ry^2 individuals to an increase in drosopterins as well as in HB pteridines (Hadorn, 1956a). In contrast with this result, we find, as shown in Figure 9b, the two groups of substances negatively correlated in ry^2 individuals, in which the drosopterins have been induced to increase by implanting + malpighian tubes (Hadorn and Graf, 1958). In interpreting this discrepancy it should be kept in mind that the temperature experiment differs fundamentally from the implantation. Only in the second set-up is xanthine dehydrogenase provided to the ry^2 system, enabling it to utilize its store of HB_1. Since lowering of temperature does not lead to formation of isoxanthopterin, a decrease in the HB fraction cannot be expected.

Non-autonomous Development of Pteridines

We have repeatedly referred to the fact that eyes of the *rosy* mutant (ry^2) manifest a wild-type phenotype with respect to drosopterins when exposed either as hosts or as implants to the influence of the ry^+ agent. Though corresponding experiments have not been carried out with *maroon-like* (*ma-l*), the behavior of this mutant in gynandromorphic mosaics (Glassman, 1957) shows that here too we are dealing

with non-autonomous development. We have already stressed that drosopterin deficiency of all other tested mutants behaves as an autonomous character. This system is paralleled by the classical studies on the brown eye pigments (ommo-

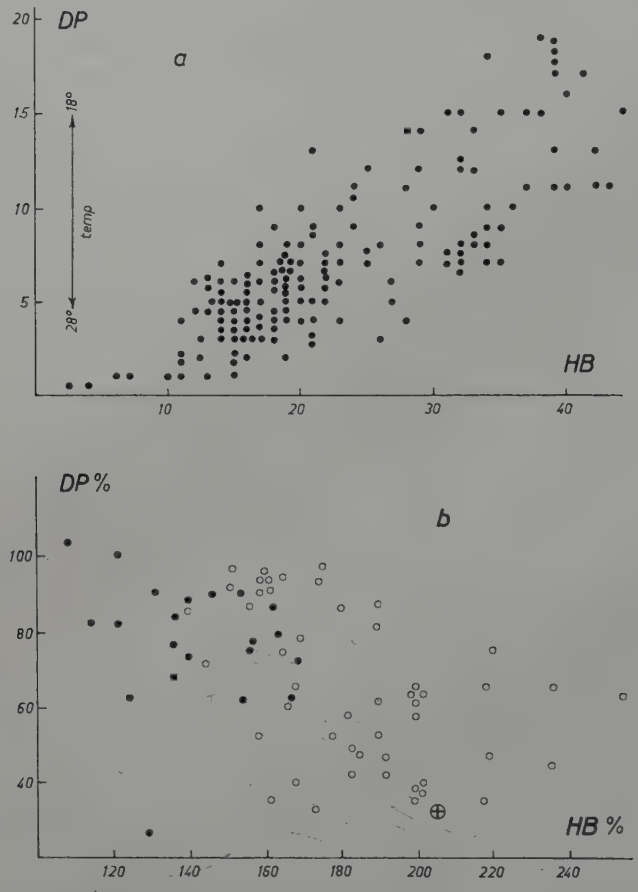


FIGURE 9. *Above*: Positive correlations between 2-amino-4-hydroxypteridine (HB fraction) and drosopterins (DP) in heads of ry^2 flies raised at different temperatures (28°–18°C.), quantities indicated in arbitrary units (from Hadorn, 1956a); *below*: negative correlations between HB and drosopterins (DP) in ry^2 heads after implantation of + malpighian tubes, quantities in percentages of wild-type controls; symbols as in Figure 7 (from Hadorn and Graf, 1958).

chromes). Only a few mutants of *Drosophila* such as *vermilion* and *cinnabar* are non-autonomous.

It follows that only a few among the gene-conditioned links of the two chains of reaction which lead eventually to drosopterins or ommochromes are of such a nature that they can be influenced, repaired, or substituted by agencies which are extrinsic to the differentiating cells. Let us compare, as an example, the mutants *sepiaoid*

(*sed*) and *rosy* (ry^2). In both we find a deficit in drosopterins of the same order of magnitude. But when transplanted into wild type, *sed* eyes, in contrast to ry^2, do not achieve normalization of their drosopterin content (Goldschmidt and Hadorn, unpublished). Apparently the two mutants interfere with biochemically different links of the drosopterin chain. The results from reciprocal experiments are difficult to explain: a + eye appears non-autonomous as an implant in a ry^2 host where it reaches only the pigment level of the mutant genotype (Hadorn and Schwinck, 1956); when implanted in *sed* hosts, however, the + eyes behave autonomously with regard to drosopterins. This difference can provisionally be interpreted as follows: any eye primordium, regardless of its genetic constitution, is dependent in its drosopterin formation on functions of other cellular systems. Therefore, a + eye remains *rosy* in a ry^2 host because the mutant environment is unable to provide the implant with such indispensable help. A *sed* host, however, would be fully capable of furnishing the necessary agent. On the other hand, the eyes of *sed* hosts as well as *sed* implants in + hosts remain deficient in drosopterins because their cellular system is impaired intrinsically by the mutation. In conclusion: ry^2 blocks a normal metabolic process which is initiated outside the eye system and therefore non-autonomy is observed; whereas *sed* and other autonomous mutations affect the metabolic competence of the ocular cells directly without interfering with the contribution which other cell systems furnish to the drosopterin formation in eyes.

The testes of *Drosophila melanogaster* represent another and even more convincing example of an organ system which is not self-sufficient in pteridine synthesis. Experimental evidence is given in Figure 10. The relevant results may be summarized as follows: (1) the sex of the host determines to a considerable extent the amount of isoxanthopterin present in implanted imaginal testes—when larval testes grow as implants in females, much smaller quantities of isoxanthopterin are formed in them than in testes developing in male hosts; (2) testes of the wild type behave completely "non-autonomously" in ry^2 hosts—they are unable to form any isoxanthopterin; (3) the ry^2 testes after developing in + hosts contain the same amount of isoxanthopterin as corresponding + implants.

Since the appearance of isoxanthopterin is dependent on xanthine dehydrogenase, it follows from results presented that this enzyme cannot autonomously arise in testes in an active form. This interpretation is the more valid because we find the precursor HB_1 highly accumulated in testes blocked in isoxanthopterin synthesis (Figure 2). On the other hand, the nature of the biochemical agent, which after penetrating from outside into the testis sheet causes the appearance of isoxanthopterin, remains obscure. Though it is rather difficult to visualize that the diffusible substance could be the xanthine dehydrogenase itself, we have at any rate to postulate a principle on which the oxidation of the HB_1 to isoxanthopterin depends directly or indirectly. From the reported experiments it is clear that ry^+ malpighian tubes and fat bodies are potent producers of such a ry^+ substance. The general dependence of isoxanthopterin content of various organs on extraneous sources makes it plausible that non-autonomous isoxanthopterin phenes may appear in any transplantation experiments in which genotypes differing in xanthine dehydrogenase activity are involved. This statement suggests that we should also expect a non-autonomous appearance of other pteridines whose quantity is directly correlated with the amount of isoxanthopterin. First of all, the precursor HB_1 should be affected. This is in fact observed in experiments such as that illustrated in Figure 7. To what extent and for what reason other pteridines of various genotypes behave non-autonomously must be determined by further studies.

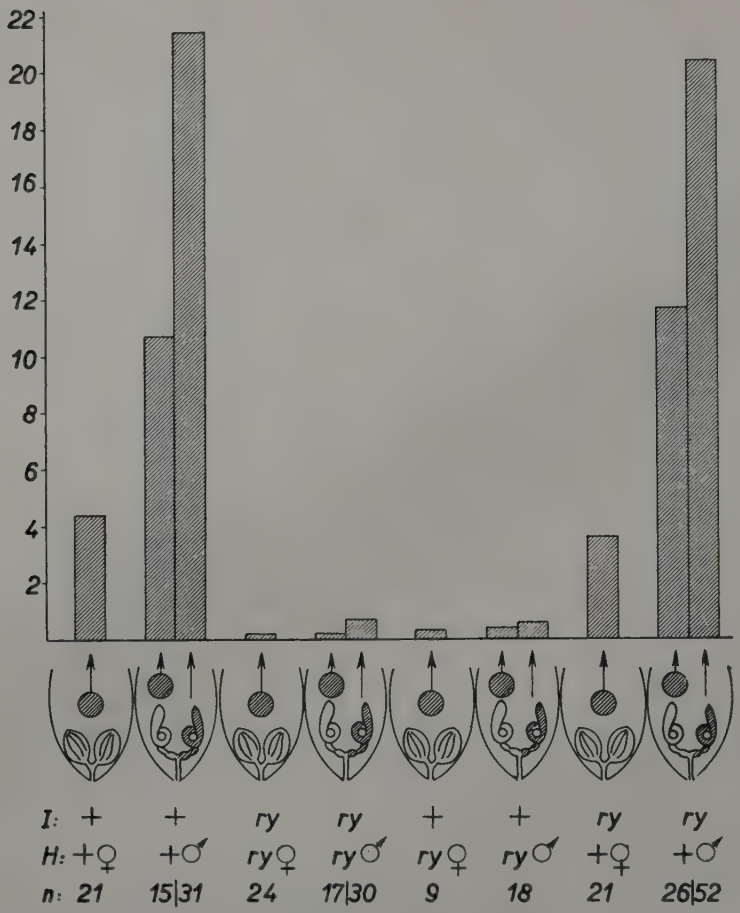


FIGURE 10. Quantities of isoxanthopterin (histograms of means) in single adult testes. The experimental set-ups are sketched at the base of the columns, each of which represents the mean of n measurements. Free testes remain smaller than attached ones. I, genotype of implant; H, genotype and sex of host; n, number of testes measured. The fluorescence in ry^2 hosts is not due to isoxanthopterin but caused by small amounts of other pteridines. From Hadorn, Graf, and Ursprung (1959).

Some recent experiments indicate that autonomy for drosopterin formation in *Drosophila* eye disks implanted into hosts of different genotypes does not necessarily imply autonomy of all other pteridines (Goldschmidt and Hadorn, unpublished). The eyes of the mutant *sepiaoid* (*sed*) are, as already mentioned, characterized by a reduction of drosopterin content to about one-third of wild type. Besides however an enormous increase of sepiapteridine and HB_2 (biopterin) is found, while isoxanthopterin appears in hardly measurable traces.

A wild-type eye transplanted into the *sed* mutant is able to reach the normal level of drosopterin, but is as deficient in isoxanthopterin as the host. It exhibits, moreover, severe disturbances in the concentration of three other pteridines: while

the increase in HB_1, the precursor of isoxanthopterin, could be expected, we are unable, at present, to offer an interpretation for the pronounced storage of isoxanthopterin, exceeding by far the content of the *sepiaoid* eye, or for the sharp drop in the sepiapterin fraction.

Isoxanthopterin as a Secondary Sex Character

An adult male of wild-type *Drosophila melanogaster* contains about ½ μg. isoxanthopterin (Robertson and Forrest, 1957; Hadorn, Graf, and Ursprung, 1958). In females of the same imaginal age we find hardly more than one-fourth of this amount. This sex difference is first of all due to the mentioned accumulation of isoxanthopterin in the testes. Ovaries lack this metabolite almost completely. But the sexual difference in isoxanthopterin becomes also clearly manifest in organs such as eyes and malpighian tubes which are otherwise not sexually differentiated. Studying the development of isoxanthopterin in eyes, we measured in males about twice the quantity in females (Hadorn and Ziegler-Günder, 1958).

By means of transplantations we tried to learn whether the isoxanthopterin difference in eyes of the two sexes is directly conditioned by the intrinsic chromosomal constitution or extrinsically caused by a sexual difference in the humoral milieu. The experimental set-ups are represented in Figure 11. Larval eye disks were implanted in male and female hosts of the same age. At this stage no pteridines are found in eye primordia. The isoxanthopterin of the implants was then measured three days later, in the second half of the pupal period, when this substance has almost reached its final and maximal concentration. It is clear that the amount of

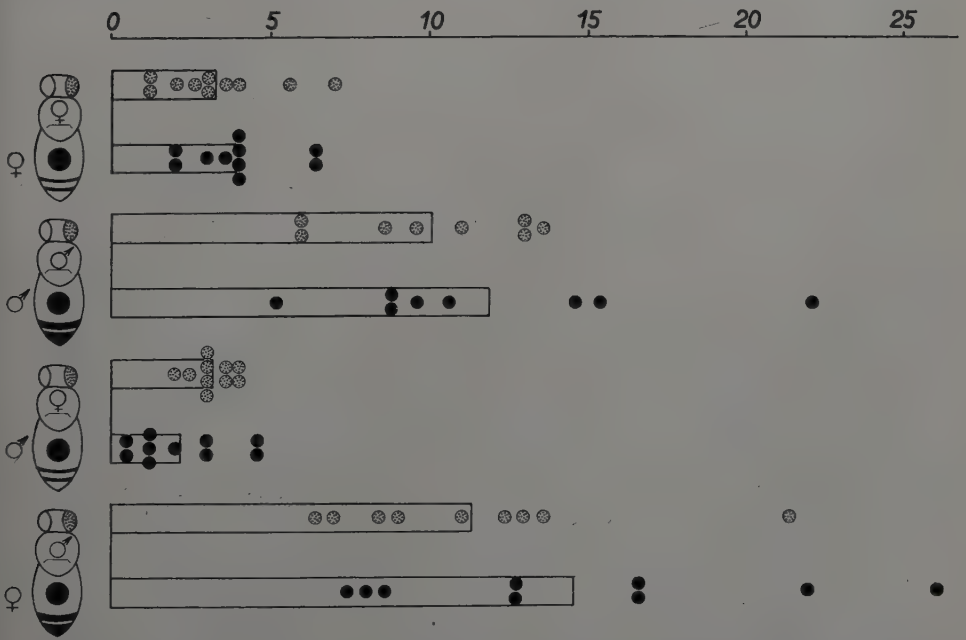


Figure 11. Quantities of isoxanthopterin in pupal eyes of wild-type *Drosophila melanogaster*. Host stippled, implanted eye black. Each circle refers to six eyes measured together. Means indicated by length of columns. The sex of the host is indicated on the thorax, that of the implant to the left of the abdomen. From Hadorn and Ziegler-Günder (1958).

isoxanthopterin of transplanted eyes corresponds exactly to the level characteristic of the host sex. The intrinsic chromosomal constitution has no influence. This non-autonomous development constitutes further evidence for the assumption that isoxanthopterin content in diverse organs depends on diffusible substances which originate outside the site where this pteridine is finally found. Physiologically the sex difference in isoxanthopterin could be based either on a higher level in xanthine dehydrogenase in males or on some other difference modifying the success of this enzyme as measured by the yield of isoxanthopterin. At any rate, the pteridine content of *Drosophila* eyes can be interpreted as a secondary sexual character of a biochemical nature.

Relations between Pteridines and Ommochromes

Pteridines and ommochromes are by no means biochemically closely related. Nevertheless, there must be some developmental connection between them. This follows from the fact that many mutations of *Drosophila melanogaster*, such as *w*, *rb*, *car*, and *bw*, affect the quantities of pteridines as well as the amount of brown eye pigments (Hadorn and Mitchell, 1951; Nolte, 1952). This relationship is even more apparent in genotypes of *Ephestia kühniella*. In this moth the red-eyed mutant *a* which behaves as homologous to *vermilion* (*v*) of *Drosophila* is deficient in ommochromes. From chromatographic studies we know that in animals of the *Ephestia* mutant *a* the pattern of pteridines is strikingly affected (Hadorn and Kühn, 1953). More compounds are observed than in the wild type and some of those which appear also in a^+ are strongly increased.

Individuals of the mutant genotype which are parabiotically connected with a wild-type partner not only form ommochromes, but also manifest the wild phenotype in their pteridine inventory (Kühn and Egelhaaf, 1955). In another experiment kynurenin was injected into *Ephestia* homozygous for *a*. As expected, by providing this precursor of ommochromes, the capacity for dark pigment formation can be restored; besides, however, the various pteridine characters develop non-autonomously, manifesting the wild-type inventory. Increase in ommochromes is thereby correlated with a decrease in pteridines.

At the present time we can hardly do more than just register this puzzling phenomenon because a biochemical link between kynurenin supply and change in pteridine synthesis is far from being understood. Kühn (1956) suggested an interesting hypothesis. From direct observation of different eye-color genotypes, he concludes that the final steps in the synthesis of ommochromes as well as of pteridines take place in RNA-containing protein granules which are present in the pigment-forming eye cells. The white-eyed mutant *wa*, which is deficient in both groups of compounds, lacks these structures. Kühn assumes that the ommochromes and pteridines might compete with regard to sites of reaction available in these granules and therefore a developmental interdependence between red and brown pigments would be observed.

REFERENCES

Beadle, G. W., and B. Ephrussi. 1936. The differentiation of eye pigments in *Drosophila* as studied by transplantation. Genetics *21*: 225–247.

Danneel, R. 1955. Ueber die beiden gelbäugigen Mutanten *sev* und *secn* der Fruchtfliege *Drosophila melanogaster*. Naturwissenschaften *42*: 566.

FORREST, H. S., E. GLASSMAN, and H. K. MITCHELL. 1956. Conversion of 2-amino-4-hydroxypteridine to isoxanthopterin in *D. melanogaster*. Science *124*: 725–726.

FORREST, H. S., and H. K. MITCHELL. 1954a. Pteridines from *Drosophila*. I. Isolation of a yellow pigment. J. Am. Chem. Soc. *76*: 5656–5658.

——— 1954b. Pteridines from *Drosophila*. II. Structure of the yellow pigment. J. Am. Chem. Soc. *76*: 5658–5662.

——— 1955. Pteridines from *Drosophila*. III. Isolation and identification of three more pteridines. J. Am. Chem. Soc. *77*: 4865–4869.

GLASSMAN, E. 1957. Studies on *maroon-like* eye color mutant. Drosophila Inform. Serv. *31*: 121–122.

GLASSMAN, E., and H. K. MITCHELL. 1959. Mutants of *Drosophila melanogaster* deficient in xanthine dehydrogenase. Genetics (in press).

GRAF, G. E., und E. HADORN. 1959. Untersuchungen über fluoreszierende Stoffe der Hoden von *Drosophila*. Zschr. Naturforsch. *14* (b): 14–17.

GRAF, G. E., E. HADORN, and H. URSPRUNG. 1959. Experiments on the isoxyanthopterin metabolism in *Drosophila melanogaster*. J. Insect Physiol. (in press).

HADORN, E. 1954a. Approaches to the study of biochemical and developmental effects of mutations. Proc. IX Internat. Congr., Genet., Caryologia *6* (Suppl.): 326–337.

——— 1954b. Ontogenetische Aenderungen im Gehalt an Isoxanthopterin bei verschiedenen Genotypen von *Drosophila melanogaster*. Experientia, Basel *10*: 483–484.

——— 1956a. Patterns of biochemical and developmental pleiotropy. Sympos. Quant. Biol. *21*: 363–373.

——— 1956b. Erbkonstitution und Merkmalsbildung. Verh. Schweiz. Naturf. Ges. Basel. *136*: 52–66.

HADORN, E., und A. EGELHAAF. 1956. Biochemische Polyphänie und Stoffverteilung im Körper verschiedener Augenfarb-Genotypen von *Ephestia kühniella*. Zschr. Naturforsch. *11*(b): 21–25.

HADORN, E., und G. E. GRAF. 1959. Weitere Untersuchungen über den nicht-autonomen Pterinstoffwechsel der Mutante *rosy* von *Drosophila melanogaster*. Zool. Anz. *160*: 231–243.

HADORN, E., G. E. GRAF, und H. URSPRUNG. 1958. Der Isoxanthopterin-Gehalt transplantierter Hoden von *Drosophila melanogaster* als nicht-autonomes Merkmal. Rev. suisse zool. *65*: 335–342.

HADORN, E., und A. KÜHN. 1953. Chromatographische und fluorometrische Untersuchungen zur biochemischen Polyphänie von Augenfarb-Genen bei *Ephestia kühniella*. Zschr. Naturforsch. *8*(b): 582–589.

HADORN, E., und R. KÜRSTEINER. 1956. Unterschiede in Exkretstoffen bei verschiedenen Genotypen von *Drosophila melanogaster*. Arch. Julius Klaus Stift. *30*: 494–498.

HADORN, E. and H. K. MITCHELL. 1951. Properties of mutants of *Drosophila melanogaster* and changes during development as revealed by paper chromatography. Proc. Nat. Acad. Sc. U.S. *37*: 650–665.

HADORN, E. und I. SCHWINCK. 1956. Fehlen von Isoxanthopterin und Nicht-Autonomie in der Bildung der roten Augenpigmente bei einer Mutante (*rosy*^{2}) von *Drosophila melanogaster*. Zschr. indukt. Abstamm. *87*: 528–553.

HADORN, E. und I. ZIEGLER-GÜNDER. 1958. Untersuchungen zur Entwicklung, Geschlechtsspezifität und phänogenetischen Autonomie der Augen-Pterine verschiedener Genotypen von *Drosophila melanogaster*. Zschr. Vererb. *89*: 221–234.

KÜHN, A. 1956. Versuche zur Entwicklung eines Modells der Genwirkungen. Naturwissenschaften *43*: 25–28.

KÜHN, A., und A. EGELHAAF. 1955. Zur chemischen Polyphänie bei *Ephestia kühniella*. Naturwissenschaften *42*: 634–635.

LERMA, B. DE, e M. DE VINCENTIIS. 1955. Identificazione chimica e valuazione biologica delle sostanze fluorescenti di *Drosophila melanogaster* Meig. Boll. Zool. *22*: 1–15.

MITCHELL, H. K., E. GLASSMAN, and E. HADORN. 1959. Hypoxanthine in *rosy* and *maroon*-like mutants of *Drosophila melanogaster*. Science *129*: 268–269.

NAWA, S. and T. TAIRA. 1954. Pterins found in silkworm and *Drosophila*. Proc. Jap. Acad. *30*: 632–635.

——— 1955. Genetical and biochemical studies on pteridine metabolism. Annual Rep. Nat. Inst. Genet. Japan *6*: 36.

NOLTE, D. J. 1952 The eye-pigmentary system of *Drosophila*. III. The action of eye-colour genes. J. Genet., Cambr. *51*: 142–186.

ROBERTSON, F. W., and H. S. FORREST. 1957. Genetic variation of isoxanthopterin content in *Drosophila melanogaster*. Univ. Texas Pub. 5721, pp. 229–237.

SAKAGUCHI, B. and M. TSUJITA. 1955. Genetical and biochemical studies on the yellow lethal silkworm. IV. Changes in quantity of pterin compounds in explanted ovaries. Annual Rep. Nat. Inst. Genet. Japan *6*: 33–34.

TAIRA, T., S. NAWA, and C. OSHIMA. 1957. Pterin dehydrogenase in eye-colour mutants of *Drosophila melanogaster*. Drosophila Inform. Serv. *31*: 164.

VINCENTIIS, M. DE. 1956. Observation on the appearance of fluorescent substances during the development of the mutant *sepia* of *Drosophila melanogaster* Meig. Ricer. Sc. *26*: 2735–2738.

VISCONTINI, M. DE. 1958a. Fluoreszierende Stoffe aus *Drosophila melanogaster*. 9. Mitteilung: Kristallisiertes Isodrosopterin. Helvet. chim. acta *41*: 922–924.

——— 1958b. Fluoreszierende Stoffe aus *Drosophila melanogaster*. 10. Mitteilung: Beitrag zur Konstitutionsaufklärung der Drosopterine. Helvet. chim. acta *41*: 1299–1304.

VISCONTINI, M. DE, E. HADORN, und P. KARRER. 1957. Fluoreszierende Stoffe aus *Drosophila melanogaster*: Die roten Augenfarbstoffe. 5. Mitteilung. Helvet. chim. acta *40*: 579–585.

VISCONTINI, M., A. KÜHN, und A. EGELHAAF. 1956. Isolierung fluoreszierender Stoffe aus *Ephestia kühniella*. Zschr. Naturforsch. *11*(b): 501–504.

VISCONTINI, M., E. LOESER, P. KARRER, and E. HADORN. 1955a. Fluoreszierende Stoffe aus *Drosophila melanogaster*, II. Helvet. chim. acta *38*: 1222–1224.

——— 1955b. Fluoreszierende Stoffe aus *Drosophila melanogaster*, III. Helvet. chim. acta *38*: 2034–2035.

ZIEGLER-GÜNDER, I., und E. HADORN. 1958. Manifestation rezessiver Augenfarb-Gene im Pterininventar heterozygoter Genotypen von *Drosophila melanogaster*. Zschr. Vererb. *89*: 235: 245.

THE ROLE OF CHROMOSOMAL LOCI IN ENZYME FORMATION

J. R. S. Fincham[1]

IN THIS PAPER I shall, first, outline some of the results which Dr. J. A. Pateman and I have obtained in our studies of the *am* locus of *Neurospora crassa,* and, secondly, consider in a more general way our present knowledge of chromosome-enzyme relations.

The *am* locus of *Neurospora crassa* seems to be specifically concerned with the enzyme glutamic acid dehydrogenase. It has been located with a fair degree of precision by Dr. R. W. Barratt in linkage group V. We now have a series of eleven independently isolated *am* mutants,[2] all of them displaying the same phenotype. The basis of this phenotype is evidently the absence of the enzyme glutamic dehydrogenase; if any of this enzyme is present in the mutants it must be at less than 0.2 per cent of the concentration found in the wild type. The mutants grow well if supplied with α-amino nitrogen in the form of any one of the several amino acids which can serve as substrates for the known *Neurospora* transaminases, and they will begin to grow on minimal medium after a fairly prolonged lag, though still without producing detectable glutamic dehydrogenase. All our earlier intercrosses of *am* mutants, with screening of what we then regarded as reasonably large numbers of ascospores, suggested that all the *am* strains were due to mutation at the same locus, though they were not all the same since Pateman (1957) was able to show differences in induced back-mutation rate between some of them. More recent work (Pateman and Fincham, 1958; Pateman, 1958) has shown that phenotypically wild-type or semi-wild ascospores can be recovered with low frequency from most crosses between strains carrying different alleles, though no wild types have ever been found among comparable numbers of ascospores from crosses between strains carrying the same allele. Some of the data are shown in Table I. The crosses which give apparent wild types are of two kinds. Crosses of *1* $\times$ *2* and *1* $\times$ *3* gave a relatively high frequency (up to 0.2 per cent) of ascospores which grew more like wild types than *am*'s on minimal medium, but which grew less well on minimal than true wilds, and produced only about 10 to 20 per cent of the typical wild-type level of glutamic dehydrogenase. Most or all of these proved to be pseudo-wilds (Pittenger, 1954) and not true wild types; on backcrossing to one or other of the parents these pseudo-wild strains proved to contain no wild-type allele. Pseudo-wilds are believed to originate as disomic ascospores in which two homologous chromosomes have been included, and their occurrence among the progeny of an inter-allele cross implies a complementary interaction between the alleles in question. I shall return to this unexpected complementarity in a later part of this paper. The second kind of cross, exemplified by

[1]University of Leicester, Leicester, England.

[2]The symbols used for the *am* mutants in this paper differ from those used in Pateman and Fincham (1958). The superscripts *32*, *47*, *29*, and *R* are replaced in this paper by *1*, *2*, *3*, and *4* respectively. Mutant *5* was supplied to us by Dr. R. W. Barratt (it was originally designated B501), and *6* and *7* by Dr. D. G. Catcheside. Mutants *8–11* were isolated at Leicester. All were induced by ultraviolet light, except *3* and *4*, which were isolated following treatment with 20-methylcholanthrene and β-propiolactone respectively.

TABLE I

RESULTS OF CROSSES BETWEEN STRAINS CARRYING DIFFERENT *am* ALLELES*

Cross	Ascospores screened (thousands)	Percentage of true wild types	Percentage of pseudo-wilds
1×*2*	156	0†	0.06†
1×*3*	184	0†	0.23†
2×*3*	651	0.002	0
2×*4*	715	0	0
1×*4*	1293	0.0008	0
3×*4*	1377	0.0002	0

*Data from Pateman and Fincham (1958).
†All that were tested were pseudo-wilds, but the presence of a small minority of true wilds not excluded.

2 × *3*, *1* × *4*, and *3* × *4*, yielded no pseudo-wilds but a very low frequency (0.0002–0.002 per cent) of ascospores which gave cultures indistinguishable, both phenotypically and in their breeding behaviour, from standard wild types. Whether true wild types are produced in the same order of frequency by the crosses which give pseudo-wilds is uncertain; the relatively high frequency of pseudo-wilds in these cases makes true wilds difficult to select for. It is clearly important to know whether the true wild types arise by orthodox crossing-over or by the mysterious process known as gene conversion or transmutation (Beadle, 1957). Table II,

TABLE II*

Cross	Ascospores screened (thousands)	Numbers of am^+ ascospores			
		sp +	++	*sp inos*	+ *inos*
sp am^3 + × + am^2 *inos*	1,214	9	0	8	7
+ am^3 *inos* × *sp* am^2 +	1,549	17	0	6	0

Linkage map: sp — am — inos.
←ca.7→←ca. 3→

*Data from Pateman (1958).

which is abstracted from a recent paper by Pateman (1958), shows an attempt to answer this question for the cross *2* × *3* by following the segregation of markers fairly closely placed on each side of the *am* locus. It will be seen that most of the ascospores which carry am^+ are not recombinant for the outside markers. Only one of the two possible types of recombinant for the outside markers was found among the am^+ ascospores, but the type of recombinant recovered was not altered by reversing the *am* alleles with respect to the markers. While the distribution of the markers seems to be affected in some way by the selection of ascospores which carry a wild-type allele at the *am* locus, it is certainly not such as would be expected on the crossing-over hypothesis. Few, if any, of the am^+ ascospores could have arisen by ordinary crossing-over although they do show a higher frequency of recombination for the outside markers than would be expected in a random sample of ascospores.

Perhaps the most interesting aspect of the situation at the *am* locus is the unexpected complementation shown by certain pairs of alleles. By obtaining all the alleles in strains which were mutually compatible as regards heterocaryon formation we were able to test all possible pairwise combinations of the eleven *am* alleles for complementarity in heterocaryons. We found that only two combinations —*1* + *2* and *1* + *3*—were complementary. These pairs of alleles gave heterocaryons which grew on minimal medium without lag, though more slowly than wild type, and yielded, in cell-free extracts, specific glutamic dehydrogenase activities in the range 40 to 150, as compared with 500 or more in wild-type extracts. Of the combinations which showed no evidence of complementation, all pairs in which *1*, *2*, *4*, or *5* are involved have been checked by forcing heterocaryosis by the use of unrelated nutritional markers and demonstrating that the resulting heterocaryons resemble *am* mutants. The alleles *4* to *11* cannot be distinguished from each other on the basis of the data which I have presented so far. That they are not all alike is shown by their behaviour in heterocaryons with what may be termed a secondary mutant allele. This allele was isolated by Pateman (1957) as an apparent back-mutation following ultraviolet treatment of a strain carrying allele *2*. Further investigation (Fincham and Pateman, 1957) showed that strains carrying this new allele, which I will call *2l*, differed from wild type in producing a qualitatively different type of glutamic dehydrogenase. The altered enzyme had very little activity as extracted at low temperature from the mycelium, but could be activated by mild heat treatment. The activation is fully reversible, and it appears that this variety of the enzyme exists as an equilibrium mixture of active and inactive forms, the position of the equilibrium depending on the temperature. Various lines of evidence (Fincham, 1957) confirmed that the unique character of the enzyme resided in the enzyme molecule itself rather than in any other component of the mutant extracts, and the argument has now been further strengthened by the approximately fortyfold purification of both wild-type and *2l* enzymes without appreciable alteration of their respective properties (unpublished experiments). The allele *2l* has been combined in forced heterocaryons with ten of the other alleles in turn, and the type of enzyme which is produced in each type of heterocaryon has been investigated. The heterocaryons *2* + *2l* produced glutamic dehydrogenase which resembled *2l* enzyme in its large response (usually a six- to twelvefold increase in the initial rate of the reaction following treatment) to brief warming, and the amount of activity was generally less than in the case of *2l* homocaryons. Thus, there is no indication of any complementary interaction between *2* and its mutant derivative *2l*. The heterocaryon *1* + *2l* gave a strikingly different result, producing glutamic dehydrogenase which was very much more active than typical *2l* enzyme before the heat treatment, but giving much less response to heat. The glutamic dehydrogenase from this heterocaryon behaves, in fact, like a mixture of wild-type and *2l*-type enzymes. The heterocaryon *3* + *2l* gives glutamic dehydrogenase activity which behaves rather like that from *1* + *2l*, though the amount of apparent wild-type enzyme formed has usually been rather less in this case (Pateman and Fincham, 1958). We thus seem to have the situation that the alleles *1*, *3*, and *2l* show complementation in all combinations. Among the other alleles *4*, *7*, *9*, and *11* resemble *1* and *3* in producing a relatively heat-insensitive enzyme in combination with *2l*, although they seem to vary among themselves in the *amount* of activity usually produced; *4* + *2l*, in particular, seems characteristically to give rather low activity which responds relatively little to the heat treatment (Fig. 1). Allele *5* resembles *2* in showing no interaction with *2l*, while *6* and *8*

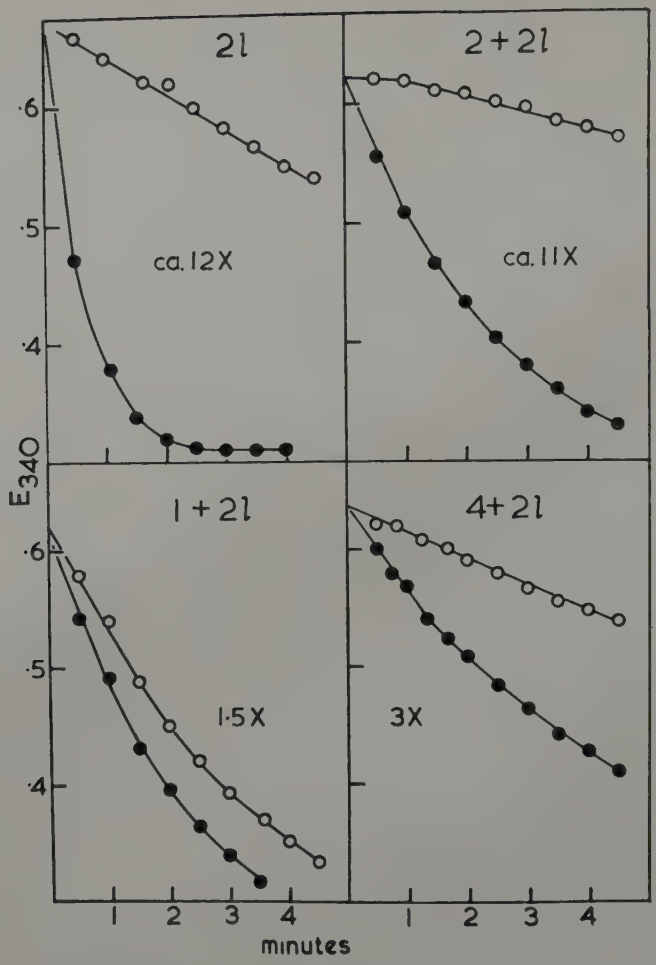


FIGURE 1. Glutamic dehydrogenase assays (measuring decrease in reduced TPN concentration at 340mμ) on extracts of a *2l* homocaryon and heterocaryons containing *2l* together with other *am* alleles. Open circles: extracts untreated; solid circles: extracts warmed to 38.5°C for 5 min. immediately before assay. All assays run at about 20°C. The factor by which the activity was increased by warming is shown in each case. The *2l* extract contained about twice as much protein (0.8 mg. per assay) as the other three extracts (each about 0.4 mg. per assay). For experimental details, see Pateman and Fincham (1958).

show only slight indications of interaction with this allele; *10* has not been tested. It seems to be simplest to suppose that the interaction between *2l* and various other alleles results in the formation of some wild-type enzyme in addition to *2l* enzyme, but it is not at all ruled out that some of the heterocaryons produce still further varieties of glutamic dehydrogenase. This possibility will have to be further investigated. The complementarity relationships of the *am* alleles are summarized in Figure 2.

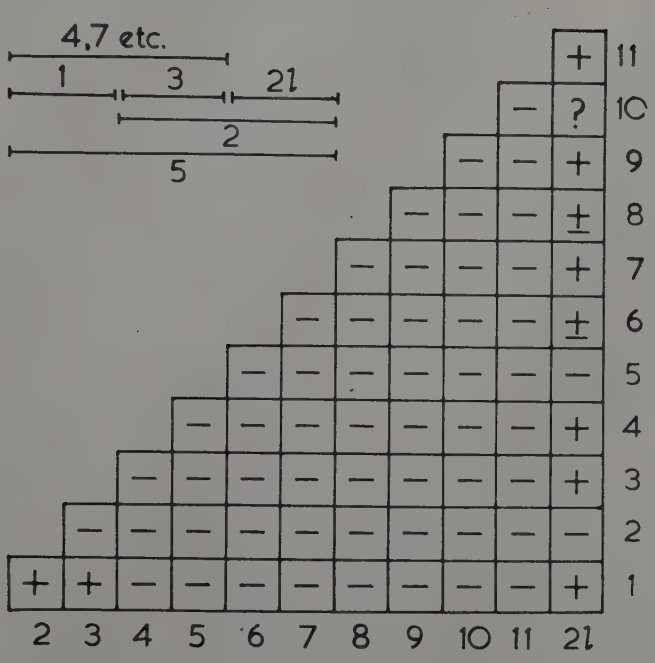


FIGURE 2. Complementarity relationships of *am* alleles in heterocaryons, with a possible interpretation in terms of effects on segments of a linear locus.

A question which may be considered at this point is whether one can consider that the chromosome segment in which the *am* mutations are located is a "locus" at all in any acceptable sense. It is, I think, clearly some kind of significant unit, yet it corresponds to none of the three kinds of genetic unit which have been proposed by Benzer (1957) and which cover so adequately the situation in bacteriophage. The *am* region must include many different mutons, since the production of wild types from intercrosses of *am* mutants, whatever its mechanism, must mean that the mutational damage is at different sites in different mutants. Whether or not orthodox crossing-over, as well as conversion, occurs within the region is not clear and is technically a difficult problem. In the *pan*-2 locus, described by Case and Giles (1958), which also shows complementation between certain alleles, it seems probable that both inter-allelic crossing-over and conversion can occur in the same cross. Thus it is likely that complex loci of this type (and one suspects that this includes most of the identified *Neurospora* loci) will not conform to the definition of a recon either. Finally, and this is probably the most disturbing feature, the *am* locus is not even a cistron, since some of its mutant derivatives are complementary in action. This inter-allele complementation seems to be not at all uncommon, at least in *Neurospora*. In addition to Case and Giles' example of the *pan*-2 locus, complementation between alleles has been reported at the *pdx* (Mitchell, 1955) and *pyr*-3 (Mitchell and Mitchell, 1956) loci. These three loci have not been associated with single enzymes, though such an association seems probable, but in two others, not including the *am* locus, information on enzymes is available. Giles *et al.* (1957), in a study which, in many respects, parallels our

work on the *am* locus, showed complementation between certain mutant alleles at the *ad*-4 locus in the formation of adenylosuccinase. Finally, I have recently been able, through the kindness of Dr. D. G. Catcheside, to study a pair of alleles at the *arg*-10 locus, which is involved in the formation of argininosuccinase (Newmeyer, 1957; Fincham and Boylen, 1957). Catcheside obtained twelve new occurrences of mutation at this locus, and he found that just one out of all the possible pairings of the twelve mutants gave a heterocaryon which grew on minimal medium. Although the parent homocaryons produce no detectable argininosuccinase, the heterocaryon does form the enzyme, though only to a concentration of 5 to 10 per cent of that found in the wild type, and is able to grow at about 10 per cent of the wild-type rate on minimal medium. This sub-wild character of inter-allele heterocaryons seems rather general. It seems probable that complementation between alleles is seldom fully efficient.

What can be the mechanism of complementary action of alleles in enzyme formation? One obvious possibility is that complementary alleles are each contributing a different enzyme to a multi-enzyme system. This seems very unlikely in our glutamic dehydrogenase case since, first, glutamic dehydrogenase activity cannot be obtained by mixing extracts of different mutants, or by extracting artificial mixtures of mycelium from different mutants, and, secondly, we have now been able to achieve up to a fiftyfold purification of *Neurospora* glutamic dehydrogenase without finding any indication that the activity depends on more than one protein. If, in fact, the glutamic dehydrogenase activity of *Neurospora* did depend on more than one enzyme it would be a considerable biochemical novelty. Rather more plausibly one might suppose that the enzyme is a complex protein consisting of more than one polypeptide chain, and that different parts of the locus are concerned in the formation of the different polypeptide components. If this were the whole explanation one would expect, as Dr. S. Brenner has suggested to me in conversation, that the locus could be analysed into discrete cistrons, one for each polypeptide. It is, in fact, possible (Brenner, personal communication), by making appropriate assumptions about the ways in which normal and mutant polypeptide components interact, to devise an interpretation of the *am* locus in these terms, but the number of assumptions required seems rather excessive. On the face of it (Fig. 2), and if one is to have a reasonably simple cistron interpretation, the *am* locus must include at least three cistrons, and all except two of the primary mutants must cover more than one cistron. Mutants which cover more than one cistron are usually suspected of being deficiencies, but *2*, at least, cannot be a deficiency since it can readily be induced to back-mutate to wild type (Pateman, 1957), and such a numerical preponderance of deletions over point mutations seems an unlikely result of ultraviolet treatment. On the whole, I think it more plausible to regard the *am* locus as a unit without internal joints and normally responsible for the production of some single product which passes into the cytoplasm and there becomes an essential part of the enzyme-forming system. This view necessitates the perhaps unattractive assumption that nuclear products of the same kind, but with different and non-overlapping defects, can somehow be brought together in the cytoplasm in such a way as to reconstitute a workable unit. There seem to be two alternatives here. On the one hand, it might be that the complementary interaction occurred between pieces of the enzyme protein itself. If this were the case, the resulting enzyme might be expected to show evidence of its compound origin in unusual properties. Recently, I have made a preliminary comparison between the glutamic dehydrogenase produced by wild type and the heterocaryon

1 + 2. The two enzyme preparations, both partially purified, proved to be quite similar in their Michaelis constants and in their stability at 55°C., though the results do suggest some relatively small differences, which will have to be checked. The other alternative would be for the complementary interaction to occur between pieces of the enzyme-forming system, perhaps between pieces of template. One might further guess that if the template consisted of ribonucleic acid, it might be able to undergo a limited degree of replication so that a complete template could be formed from two defective pieces. Such a mechanism would account for the formation of apparently normal enzyme in the heterocaryons. The difficulty is, of course, in obtaining evidence for or against such speculations.

I would like to conclude with a consideration of the present status of the one gene–one enzyme hypothesis. In recent years there seems to have been a certain amount of disillusionment about the one-to-one idea, and, indeed, one is sometimes given the impression that the hypothesis received a decent burial around the year 1951. This, I think, is partly due to the finding of several nutritional mutants in which the basis of the metabolic defect was complicated and not easily analysable in terms of effects on enzymes, and partly to a growing realization that critical evidence for or against the hypothesis as a *general* proposition is virtually impossible to obtain. Nevertheless, the one-to-one concept is still with us, and is even, under the more recent slogan of "one cistron–one polypeptide chain," being taken up with renewed enthusiasm (cf. Crick, 1958). Restricting attention to those cases where the enzymatic basis of the mutant phenotype has been worked out, the question of interest is the degree of specificity in the relation between chromosomal loci and specific enzymes. In the first place one may ask whether single mutations each affect only one enzyme. On the whole this does seem to be true. There are a good many examples (for references, see Fincham, 1959) of mutations, both in *Neurospora* and in *Escherichia coli,* which lead to the loss of only one enzyme in a metabolic pathway, the others being untouched. A number of multiple nutritional requirements have been shown to be due, not to the loss of more than one enzyme, but to a single block in the synthesis of a common precursor, as in mutants of *E. coli* which cannot make aromatic rings (Davis, 1955), or to the loss of a single enzyme which functions in two different syntheses, as in mutants requiring isoleucine plus valine (Myers and Adelberg, 1954). An exceptionally well-documented case of the loss of two enzyme activities through a single revertible mutation, recently reported for *E. coli* by Yanofsky and his colleagues (Lerner and Yanofsky, 1957; Yanofsky and Stadler, 1958), can be interpreted either as the loss of a single protein having two enzyme activities (concerned with sequential reactions in tryptophan synthesis), or as the loss of two separate, but immunologically closely similar enzymes. Even if the second interpretation is correct it still seems likely that the locus involved has a single function in the production of a protein which serves as the immediate precursor of both enzymes. The other question is whether a given enzyme has its specificity determined by only one locus. There are, in fact, a number of cases in *Neurospora* which tend to support this idea. For instance, Giles *et al.* (1957) found that nineteen independently isolated mutants lacking adenylosuccinase were all due to mutation within the same locus, that is to say, at sites which were extremely closely or completely linked. Yanofsky and Bonner (1955) showed that twenty-four mutations, all resulting in the loss of tryptophan synthetase, or in an alteration in this enzyme (Suskind and Kurek, 1957), were all at the *td* locus. In this case there is the added complication that suppressor mutations at various other loci can partially restore enzyme activity in certain

of the *td* mutants; nevertheless it seems reasonable to suppose that the *td* locus is more specifically concerned with tryptophan synthetase production than are the various suppressor loci. In glutamic dehydrogenase all twelve of the mutations which have been found to affect this enzyme have been located within the same very short chromosome region. Work on enzymes in the arginine (Fincham and Boylen, 1957; Newmeyer, 1957) and histidine (Ames, 1957) biosynthetic pathways lends support to the generalization that mutations affecting the same enzyme are at the same locus, and mutations affecting different enzymes are at different loci. I think that the evidence from these examples is sufficiently impressive to encourage one to find excuses for those cases, of which a number certainly exist (Fincham, 1959), where more or less drastic reductions in the production of one enzyme can be brought about by mutations at any one of several different loci. Several kinds of excuse are possible, especially where the production of the enzyme in question is critically dependent on environmental conditions. The discovery of permeases and their genetic control (Cohen and Monod, 1957) gives us an entirely new set of reasons for expecting the genetic basis of induced enzyme formation to be complicated. It is probable, however, that no one has ever wanted to defend a hypothesis that an enzyme can vary *quantitatively* through mutation at only one locus. The idea to which those of us who are optimistic continue to cling is that the *specific structure* of each protein is determined by a single locus, presumably through the determination of the amino acid sequence (Crick, 1958). This hypothesis has the merit of being disprovable, since, as examples of qualitative genetic alterations in proteins accumulate, it should be possible to find examples of structural alterations of the same protein due to mutations at different loci if the hypothesis is false.

The picture which seems to be emerging, at least from the *Neurospora* work, is of chromosomes segmented into small and apparently quite sharply distinct regions, each concerned, though almost certainly indirectly, in the formation of a particular kind of protein molecule. Within these regions, which I have been calling loci, mutations can occur at many different sites. Different mutant derivatives of the same locus, that is to say alleles, can often interact at meiosis, either by crossing-over in the orthodox sense or by "conversion," to give a wild-type allele, and their cytoplasmic products can sometimes interact to give a wild or semi-wild phenotype in a heterocaryon. Loci of this kind conform to none of the usual definitions of the gene. Yet the most striking and incontrovertible fact about the chromosome as a genetic structure has surely always been its segmentation into regions of highly specific and differentiated function. It is this fact, rather than any segmentation of the chromosome with respect to crossing-over, which has given solidity to the idea of the gene, and which still does so. While the recon becomes vanishingly small, and the cistron tends to fall apart, the gene locus, regarded as a physiologically differentiated segment, still retains a semblance of reality.

REFERENCES

AMES, B. N. 1957. Enzymes of histidine biosynthesis in histidine-requiring mutants of *Neurospora*. Fed. Proc., Balt. *16*: 145. (abstract)

BEADLE, G. W. 1957. The role of the nucleus in heredity. *In* A Symposium on the Chemical Basis of Heredity, eds., W. D. MCELROY and B. GLASS, pp. 3–22. Baltimore: The Johns Hopkins Press.

BENZER, S. 1957. The elementary units of heredity. *In* A Symposium on the Chemical Basis of Heredity, eds., W. D. MCELROY and B. GLASS, pp. 70 93. Baltimore: The Johns Hopkins Press.

CASE, M. E., and N. H. GILES. 1958. Evidence from tetrad analysis for both normal and aberrant recombination between allelic mutants in *Neurospora crassa*. Proc. Nat. Acad. Sc. U.S. *44*: 378–390.

COHEN, G. N., and J. MONOD. 1957. Bacterial permeases. Bact. Rev., Balt. *21*: 169–194.

CRICK, F. H. C. 1958. On protein synthesis. Sympos. Soc. Exp. Biol. *12*: 138–163.

DAVIS, B. D. 1955. Biosynthesis of the aromatic amino acids. *In* A Symposium on Amino Acid Metabolism, eds., W. D. MCELROY and B. GLASS, pp. 799–811. Baltimore: The Johns Hopkins Press.

FINCHAM, J .R. S. 1957. A modified glutamic acid dehydrogenase as a result of gene mutation in *Neurospora crassa*. Biochem. J., Lond. *65*: 721–728.

——— 1959. The biochemistry of genetic factors. Annual Rev. Biochem. *28* (to be published).

FINCHAM, J. R. S., and J. B. BOYLEN. 1957. *Neurospora crassa* mutants lacking argininosuccinase. J. Gen. Microb., Lond. *16*: 438–448.

FINCHAM, J. R. S., and J. A. PATEMAN. 1957. A new allele at the *am* locus in *Neurospora crassa*. J. Genet., Cambr. *55*: 456–466.

GILES, N. H., C. W. H. PARTRIDGE, and N. J. NELSON. 1957. The genetic control of adenylosuccinase in *Neurospora crassa*. Proc. Nat. Acad. Sc. U.S. *43*: 305–317.

LERNER, P., and C. YANOFSKY. 1957. An immunological study of mutants of *Escherichia coli* lacking the enzyme tryptophan synthetase. J. Bact., Balt. *74*: 494–501.

MITCHELL, M. B. 1955. Aberrant recombination of pyridoxine mutants of *Neurospora*. Proc. Nat. Acad. Sc. U.S. *41*: 215–220.

MITCHELL, M. B., and H. K. MITCHELL. 1956. Tests for non-allelism at the pyrimidine-3 locus of *Neurospora*. Genetics *41*: 319–326.

MYERS, J. W., and E. A. ADELBERG. 1954. The biosynthesis of isoleucine and valine. I. Enzymatic transformation of the dehydroxy acid precursors to the keto acid precursors. Proc. Nat. Acad. Sc. U.S. *40*: 493–499.

NEWMEYER, D. 1957. Arginine synthesis in *Neurospora crassa*: Genetic studies. J. Gen. Microb., Lond. *16*: 449–462.

PATEMAN, J. A. 1957. Back-mutation studies at the *am* locus in *Neurospora crassa*. J. Genet., Cambr. *55*: 444–455.

——— 1958. Aberrant recombination at the *am* locus in *Neurospora crassa*. Nature, Lond. *181*: 1605–1606.

PATEMAN, J. A., and J. R. S. FINCHAM. 1958. Gene-enzyme relationships at the *am* locus in *Neurospora crassa*. Heredity *12*: 317–332.

PITTENGER, T. H. 1954. The general incidence of pseudo-wild types in *Neurospora crassa*. Genetics *39*: 326–342.

SUSKIND, S. R., and L. I. KUREK. 1957. Enzyme-inhibitor complex in a tryptophan-requiring mutant of *Neurospora crassa*. Science *126*: 1068–1069.

YANOFSKY, C., and D. M. BONNER. 1955. Gene interaction in tryptophan synthetase formation. Genetics *40*: 761–769.

YANOFSKY, C., and J. STADLER. 1958. The enzymatic activity associated with the protein immunologically related to trytophan synthetase. Proc. Nat. Acad. Sc. U.S. *44*: 245–253.

IMMUNOGENETICS

Ray D. Owen[1]

WITH IMPORTANT EXCEPTIONS, immunogenetics generally relies on reactions of serum antibody for identification and study of inherited characteristics. In some of the most studied systems, these antibodies are normally present in the serum. More versatile tools are provided by *immune* antibodies, capable of reaction with antigens similar to those that induced the formation of these modified globulins. The physiological and biochemical bases for the remarkable adaptive response of immune antibody formation are still a matter of rather free speculation. There is reason to believe that an antigen is capable of making a heritable impression on stem cells from which antibody-producing cells arise, in clones, by repeated division (Coons, 1956). Whether this directed mutation-like change is cytoplasmic or nuclear, "nucleic," or "epinucleic," (Lederberg, 1958) has not been decided yet; the process may, in fact, involve effects at all these levels ((Burnet, 1956). A logical scheme suggesting that antigen has first a nuclear effect that renders the cell inducible, and second a cytoplasmic one in which antibody is actually produced and liberated by the cellular progeny of the induced stem cell, finds some support in the general field of protein biosynthesis (Schweet and Owen, 1957). An alternative is that antigen acts only to stimulate or select syntheses that are already spontaneously available, either on a molecular (Ehrlich, 1900; Jerne, 1955; Talmage, 1958) or a cellular (clonal) (Burnet, 1959) basis. The two lines of argument are not necessarily mutually exclusive; it is possible that antigen may induce heritable, clonal variation, but that clonal selection thereafter molds the antibody response and affects the retention of the organism's ability to give a secondary response upon later re-exposure to the antigen. In any case, it should be clear that the relatively new part of the field of immunogenetics that deals with the antibody response (and its abrogation, particularly in the field of "immunological tolerance" (Billingham, Brent, and Medawar, 1953; 1956; Owen, 1957)) offers challenging problems and exciting prospects for the physiological geneticist concerned with differentiation and development.

In the past, immunogenetics has dealt mainly with the genetic control of antigenic characteristics rather than with the antibody response. Serological tests have permitted identification of many inherited similarities and differences among the individuals of several species and of many species differences as well. These characteristics have been assigned symbols and have been subjected to standard genetic manipulation and interpretation. Because the material meaning of these symbols is usually uncertain, it is desirable that we consider the tools of immunogenetics and the symbols to which they lead.

Table I illustrates a simple kind of test pattern frequently encountered. We shall take this for the moment as representing the reactions of a set of red-cell samples from four different individuals, each tested against an antiserum produced by inject-

[1]Division of Biology, California Institute of Technology, Pasadena, Calif., on leave, 1957–8, to Biology Division, Oak Ridge National Laboratory (operated by Union Carbide Corporation for the U.S. Atomic Energy Commission), Oak Ridge, Tenn., U.S.A.

ing the cells of sample 1 into a rabbit. The cells of all four individuals react to this antiserum. When the serum is absorbed by the cells of individual 1, against whom the antiserum is directed, all the antibodies are removed; none remains in the absorbed fluid to react with the cells of any of the four individuals. Absorption by the cells of individual 2 removes the antibodies with which these cells react but leaves reactions for the other three individuals; and a similar situation is found for individuals 3 and 4.

As a symbolic representation of this simple test, the serologist would be likely to assign a letter, say C, to an "antigen" recognized by antibodies in this antiserum and common to the cells of individuals 1 and 2 but absent from those of individuals 3 and 4. Thus, absorption with either cells 1 or 2 removes anti-C from the antiserum, and the cells of individual 2 fail to react after such absorptions. Absorption with cells of individuals 3 or 4 leaves the anti-C antibodies in the reagent because these individuals lack C. Similarly, one might discern an antigen D, common to individuals 1 and 3 and absent in the other two, and an antigen E, found in 1 and 4. Individual 1, therefore, becomes CDE; individual 2 is C, 3 is D, and 4 is E. Of course, this classification might be only the beginning of a complete analysis of this system.

Figure 1 shows a test system with a distribution of reactions identical to that just discussed. It throws a different light on our picture because, in this case, the chemical specificities on which the reactions are based are known. Landsteiner (Landsteiner and Van der Scheer, 1936), by combining simple haptenic groups with protein-carriers, was able to discern serological reactions that depended on known chemical additions to the immunizing and test antigens. In the instance illustrated, metanilic acid diazotized to horse serum was used as the immunizing antigen, and this hapten and other similar ones were diazotized to chicken serum for the test antigens, and to sheep red-cell stromata for absorptions. You will note that the antiserum is not, in fact, completely specific for the chemical grouping that directed antibody formation; it cross-reacts with haptens in which the sulfonic acid group is in the ortho rather than the meta position or in which arsenic or benzoic acid substitutions are made for the sulfonic acid grouping in the meta position. When such an antiserum is absorbed by this set of related materials, antibody cross-reactive with each of these haptens is removed, to leave residual antibodies still reactive with the others. Only the hapten that directed the formation of this heterogeneous antibody population reacts with all its fractions.

Comparing Table I and Figure 1 we can discern a basis for serious reservation concerning the relation between the patterns of cross-reaction of antigens and details of similarity or difference in their structure. The symbolism CDE for cells of type 1 in Table I, suggesting that the cells include three antigens, each shared

TABLE I

ABSORPTION ANALYSIS OF AN ANTISERUM TO RED BLOOD CELLS OF INDIVIDUAL 1

Test cells	Serum unabsorbed	Serum absorbed by			
		1	2	3	4
1	+	0	+	+	+
2	+	0	0	+	+
3	+	0	+	0	+
4	+	0	+	+	0

ANTIMETANILIC ACID–AZO–HORSE SERUM

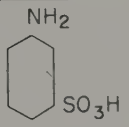
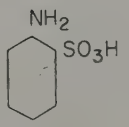
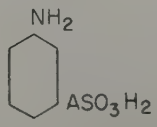
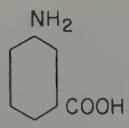
TEST ANTIGENS (–AZO–CHICKEN SERUM)	UNABSORBED	ABSORBED BY AZOSTROMATA (1)	(2)	(3)	(4)
(1) METANILIC 	+	0	+	+	+
(2) o–AMINOBENZENE SULFONIC 	+	0	0	+	+
(3) m–AMINOBENZENE ARSENIC 	+	0	+	0	+
(4) m–AMINOBENZOIC 	+	0	+	+	0

FIGURE 1. Absorptions and tests of an antiserum to "metanilic acid-azo-horse serum" (after Landsteiner and Van der Scheer, 1936).

with one of the other three individuals, cannot be related to the true character of the immunizing cells or their antigens. The symbols actually stand for fractions of *antibody* in a heterogeneous molecular population. In Figure 1, we note a fraction of this population peculiarly reactive with metanilic and o-aminobenzenesulfonic acid, removed by absorption with either of these haptens; this antibody fraction may be symbolized as anti-C. Similarly, we can discern antibody fractions that could be symbolized anti-D and anti-E. But these have no necessary relation to any detail of structure in the test antigens. E, for example, cannot stand for a carboxy substitution in the meta position, because a sulfonic acid substitution also reacts with it; in fact, it was a sulfonic acid that induced the anti-E antibodies. Neither can it stand simply for a meta substitution, because a meta substitution of arsenic acid does not produce the specific reactivity to which we might assign the symbol E. The symbols we have used represent only a shorthand way of saying that the cells of individual 2 are *similar,* in some respect, to those of individual 1 because they react with antibodies induced by 1. Individuals 3 and 4 also have something in common with 1, but they are different from each other and from 2, so we arrive at different symbols for them—D and E, respectively. But this does not mean that the cells of individual 1, which induced all these antibodies, therefore include three separate and distinguishable antigens. A single simple hapten would be subject to equally complex representation on such a basis, and its apparent complexity would be limited only by the number of other haptens at hand sufficiently similar to cross-react with separable fractions of the complex antibody population it induced and by the complexity of that population.

Now, of course, the gap between a simple hapten and a cell surface as an antigen may be great. We do not at present know completely the chemical basis for the specificity of any of the red-cell reactions with which immunogeneticists are concerned, but there can be little doubt that a number of diverse molecular types are endowed with antigenic specificities. In general, the ability to *induce* immune-serum antibody is a property of large molecules, although specific *reactions* to antibodies depend on rather small sites on these molecules (Kabat, 1957). On a molecular basis, we can distinguish three levels of similarity and difference that may find expression in the symbols of the immunogeneticist.

First, genetic variation may be concerned with the presence, absence, or extreme modification of whole macromolecules. In general, we might expect that different and clearly separate genes would affect the antigenic specificities of different molecules. For example, the alleles at the *ABO* locus probably affect different molecular species than do the alleles at the *Rh* locus in human blood. But there are clear contradictions to this as a valid generalization; several independent loci besides *ABO* affect the specificity of the soluble blood-group substances of human secretors, probably affecting the same macromolecules, and the molecules bearing the *ABO* specificities in the secretions are probably quite different from those that show the impress of the same genes, on the red-cell surface. More than one locus, therefore, may affect an antigen, and a locus may affect more than one antigenic molecule.

Second, genetic variation may be concerned with haptenic substitutions at different sites on a given macromolecular species. For example, a glycoprotein may be subject to the addition of different sugar groups in different parts of its structure. We cannot now specify with confidence any unique kind of genetic control concerned with such intramolecular variation among antigens. We might in the future find instances in which this kind of variation among the macromolecules of a species is related to closely associated genes concerned with joint or sequential elaboration of a complex nuclear product, or to modifications within a cistron, and therefore to the "fine structure" of the gene. But close physical association does not seem to be a necessary condition for genetic units affecting the final properties of the same macromolecule. Again, there is reason to believe that the alleles at the *Lewis* locus, the *secretor* locus, the *ABO* locus, and probably other loci may all be concerned with regulating the final specificity of the same macromolecular species in human secretions (Ceppellini, 1958). Somewhat similarly, alleles *A* and *B* in an *AB* human heterozygote seem to affect the same ultimate molecular antigen in the secretions (Morgan and Watkins, 1956), and allele *r'* at the *Rh* locus modifies the Rh_0 specificity produced by a heterozygote with any *R* allele, but *r* does not (Ceppellini, 1952). Such interactions, interallelic and intergenic, together with the "hybrid specificities" long known in species hybrids (Irwin and Cole, 1936) and more recently defined for a remarkable intraspecific locus in the rabbit (Cohen, 1956), suggest reactions occurring at some "distance" from the gene and becloud any picture that would show whole macromolecular antigens assembled *in situ* at the chromosomal surface and springing fully formed from complex chromosomal sites. Such syntheses *may occur* at this level, but they cannot be considered necessary and sufficient for the production of all antigens or antigenic specificities; in fact, there is, at present, no compelling evidence in this field that they do occur.

Third, "antigens" that appear to be complex, requiring strings of symbols for representation of their patterns of reactions, may, as we have seen, vary only at a particular haptenic site, their apparent complexity deriving only from the complex

patterns of cross-reaction possible to heterogeneous antibody populations. Genetic control of this kind of antigenic variation would relate an allele to the synthesis of a unique haptenic group, which might ultimately become part of the structure of one or more macromolecular species. Allelic alternatives would control similar haptens, distinguishable by antibodies but displaying patterns of similarities as well as differences when a series of them were available for cross-comparison. This interpretation of genic effects would be similar to that emerging from current studies of the chemistry of human hemoglobins, where substitution of a particular amino acid at one site in the hemoglobin half-molecule (valine or lysine for the glutamic acid of normal adult hemoglobin, in hemoglobins S and C, respectively) produces gross changes in macromolecular specificity, apparently under the control of multiple alleles (Ingram, 1957; Itano and Singer, 1958).

Short of a complete chemical characterization of the antigens involved in a given set of serological reactions, the serologist finds it difficult or impossible to distinguish among these three types of molecular bases for similarities and differences in so complicated a structure as the red cell. Genetic interpretation of these differences must depend on genetic analysis. As we have seen, we are on insecure ground if we attempt to go directly from symbols for antibody fractions to conclusions about details of antigenic structure. We compound this insecurity if we attempt to relate the "factorial formula" of an "antigen" to the structure of the gene that seems to control it, without genetic evidence to support and test our interpretations on the genetic level.

A rather regular progression has occurred repeatedly in the history of this subject. First, a *difference* is observed by which the individuals of a species can be classified into two groups, on the basis either of an antiserum prepared in one individual against the cells of another of the same species or of an antiserum produced by another species, such as the rabbit, against the cells of individuals of one type and absorbed by individuals of the second type. If the cells of each individual of the positive type absorb all the antibodies for all other positive cells, the difference is regarded as a simple serological alternative, and genetic observations generally lead to the conclusion that this alternative is controlled by a single Mendelizing unit. The alternatives are often represented by a symbolism such as *A* for the positive and *a* for the negative allele. Next, immunizations of *A* animals with *a* cells may produce "anti-a"; *a* is therefore not a void, but is a difference capable itself of inducing antibodies. With infrequent exceptions, both alleles are expressed in the heterozygote. The heterozygote often reacts more weakly or slowly to either test reagent—the "dosage effect" suggesting that more antigenic sites are produced or effectively exposed on the cell surface when there are two alleles present corresponding to a given test specificity than when there is only one. The symbolism here may progress to designating the "antigens" as A_1 and A_2, and the corresponding alleles as A^1 and A^2. We have, at this point, no way of recognizing *similarities* between the "antigens" A_1 and A_2; our techniques have been restricted to the recognition that they are different from each other.

The serological unity of the test reagents and the all-or-none alternative nature of the antigenic specificities are challenged when a related variant is identified. In cattle, for example, F and V remained simple alternatives, giving simple dosage differences with anti-F reagents distinguishing *FF* from *FV* cells, until a specificity V_2 was identified in the cells of all American bison. V_2 is similar to cattle V because it absorbs antibody to V from the anti-V reagents. Careful absorptions with bison cells fractionate these reagents, leaving antibody reactive with cattle V (now "V_1"),

not with V_2. But V_2 is similar also to F; cattalo (bison-cattle hybrids) of genotype FV^2 show the dosage effect characteristic of *FF*, not FV^1, cattle (Owen, Stormont, and Irwin, 1958).

The "three-allele" stage of knowledge of a locus is usually short-lived and is often skipped. More commonly (returning to our earlier and more general symbolism), an antibody reagent is found that reacts with some A_1 cells and not with others and with some A_2 cells and not with others. This could be, and usually is, an independent antigenic specificity. Often enough, however, it turns out that the frequencies of positives and negatives for the new "alternative" are not similar in the A_1 and A_2 classes; there may, for example, be more or fewer individuals positive for both A_1 and the new reagent than would be predicted on the basis of independence of classification. Such statistical associations generally provide the serologist with his first indication that he is dealing with a set of related specificities. The availability of another antiserum has provided a tool for recognizing similarities as well as differences between A_1 and A_2. The symbolism at this point generally reflects this situation by representing the possible "antigens" in the set as something like A_1S, A_1s, A_2S, and A_2s, and the new reagent as anti-S. The search is then on for an "anti-s," and it is not unlikely that one will be found. The quality of an individual's antibody response is affected by both his own antigenic constitution and the nature of the stimulating antigen, and the characteristics of an antiserum are modified by many other variables, including such aspecific factors as the duration and amount of the antigenic stimulus and the route by which the antigen comes into the recipient. Among the many diverse antisera in the system we have been discussing, therefore, one is likely to turn up, in time, which gives a pattern of reactions reciprocal to that of anti-S. Thus a phase is reached at which we seem to be dealing with *two pairs* of alternatives controlled by a genetic unit. The unsuspecting geneticist who takes the symbols in these formulae as representing material identities and differences is likely to start talking about pseudo-alleles or a cistron, even in the complete absence of genetic evidence for subdivisibility of the genetic unit.

With further experience, it generally turns out that A_1 and A_2, or S and s, are not really alternatives at all. For example, a new antibody reagent appears, and is labeled anti-U, which fails to react to antigens of this set only when S and s reactions are *both absent*. This suggests a series S, s, u, controlled by "alleles" *S*, *s*, S^u, in which the anti-U antibodies react with S and s alike, while individuals of genotype S^uS^u fail to react with anti-S, anti-s, and anti-U. More reagents appear, which require the addition of numerous superscripts to distinguish variants at a presumed locus as illustrated for the postulated *C* locus in the Rh complex in Table II. These variants show patterns of cross-reaction that can hardly be escaped as indications of series of related and overlapping specificities, as illustrated for the absorption analysis with C and C^w in Table III. Most "c anti-C" sera seem (Wiener and Wexler, 1956) to crossreact completely with C^w. Other antibody reagents turn up to cross hypothetical locus lines, so that, as in Table IV, when many "anti-C + D"-reactive sera in the Rh system are absorbed by cD cells, the "anti-C" is removed from the antiserum. One wonders, of course, why c cells should remove antibodies that are supposed to distinguish between C and c by *failing* to react with c, and then he may realize that the difficulty is with the tools that the symbolism provides for thinking about the situation. As shown in the lower half of Table IV, a different symbolism, based on a recognition of unitary specificities in cross-reacting systems, suggests testable interpretations easily compatible with the observations. In an anti-

TABLE II

CROSS-REACTIONS FOR ANTISERA IN THE "C SERIES" OF THE RH COMPLEX*

	Antisera							
Cells	1	2	3	4	5	6	7	8
C^u				+			+	
C^w			+	+		+	+	+
C		+	+	+	+	+	+	
c^v	+	+	+	+				
c	+							

*After Race and Sanger (1954, p. 186).

TABLE III

ABSORPTIONS OF ANTISERA PRODUCED BY "cc" PERSONS, AGAINST "C" BLOOD, IN THE RH COMPLEX*

Tested against	Serum unabsorbed	Serum absorbed by		
		C	C^w	c
C	+	0	0	+
C^w	+	0	0	+
c	0	0	0	0

*After Race and Sanger (1954, p. 183).

TABLE IV

ABSORPTIONS OF CERTAIN ANTISERA REACTING AS "ANTI-C + D," FOR THE RH COMPLEX*

Test cells	Serum unabsorbed	Serum absorbed by	
		cD	Cd
cD	+	0	+
Cd	+	0	0

rh-Anti-Rh_1 Serum?

Test cells	Serum unabsorbed	Serum absorbed by	
		Rh_0	rh′
Rh_0	+	0	+
rh′	+	0	0

*After Race and Sanger (1954, p. 189).

serum of this sort, we do not seem to be dealing with antibodies to C and D but with antibodies, probably produced against Rh_1 cells by an Rh-negative individual, essentially completely cross-reactive with Rh_0 but only partly cross-reactive with rh′ cells.

I cannot, here, advance further into the lore of this part of the field of immunogenetics. The very extensive studies of cattle red-cell reactions (Stormont, 1955), and similar work with chickens (Briles, McGibbon, and Irwin, 1950), serve as

examples of the difficulties that would arise with increasing knowledge of a system of blood-group specificities if these were regularly to be interpreted as the result of one allele–one "antigen"–one antibody relations. The assumption of close or even complete genetic linkage of such postulated alleles to explain the genetic unity of complexes does not resolve these difficulties. A sound interpretation will depend on a realistic conception of the immunochemical basis for serological reactions. The term "antigen" generally applies to the macromolecule that induces antibody formation and that reacts with antibodies in some detectable fashion. But the immunogeneticist at present usually operates at another level. In red-cell reactions, particularly, he knows little or nothing about the macromolecular character of his material, and his "antigens" are studied only for the much smaller sites within them that regulate the specificity of individual antibody molecules and react with them. Workers with soluble antigens, when they can purify their preparations, can work at the macromolecular level, and it is probably no accident that it is with such materials that deviations from a "one gene–one antigen" principle have become most clearly evident. We can still speak, in general, of "one allele–one specificity" at the antigen-antibody reaction level throughout the field, but here we need further to recognize the immunochemical fact of antibody heterogeneity. A relation "one allele–one specificity–heterogeneous antibodies" would appear at present to encompass most of the facts at hand. In general, this relation requires postulation of genetic subdivisibility for complex patterns of reactions only when genetic evidence of such divisibility is obtained.

Certain principles seem to be reasonably well established. When sets of serological reagents are available for evaluation of similarities as well as differences among groups of antigenic specificities, then similar specificities generally turn out to be inherited as though they were controlled by a set of multiple alleles—which is perhaps only another way of saying that they are controlled by similar genes. Reciprocally, sets of genes that behave in inheritance as though they are multiple alleles, when they control recognizable antigenic specificities, turn out to control related specificities. Proof that some of these specificities may relate to different parts of a gene or to different adjacent genes must await the marshalling of competent genetic evidence of the separability of postulated loci, or of events like gene conversion within heterozygotes for single genes. On serological grounds alone, symbolic complexity *need not* relate to spatially separate subgenic sites or to separate genes. Population frequency considerations provide no sound guide to the kinds of genetic events that produce the sort of variation we have been considering. As long as selection molds populations, the mutational or recombinational origins of variants are not likely to be reflected faithfully in their quality or frequency in the structure of evolving populations. Fisher (1953), for example, has set up special conditions for the Rh locus: " . . . in European conditions (though not in Africa) at least four of the combinations are being steadily eliminated by selection."

There are two forms in which closely linked genes (though not so closely linked as to justify the application of the term "pseudo-alleles") seem clearly to affect antigenic specificities. One is in the chicken, where Scheinberg (1956) has reported two independent linkage groups involving blood-group genes with recombination frequencies of 5.4 $\pm$ 4.2 per cent and 2.6 $\pm$ 2.0 per cent, respectively. The other is the *H-2* region in the ninth chromosome of the mouse, which several years ago was recognized (Snell, Smith, and Gabrielson, 1953) on histocompatibility grounds to involve two "components," d and k, and which has since seemed to be

fractionated, probably by ordinary crossing-over, in something of the order of 1 per cent of the gametes of double heterozygotes. Antigenic specificities controlled by this region are recognized by red-cell serology as well as by histocompatibility techniques (Gorer, 1956), and the batteries of reagents now available rival those for the *B* and *C* loci of cattle and the *Rh* locus of human blood for the diversity they recognize. A few apparent recombinations for this region have been reported (Allen, 1955; Hoecker, Counce, and Smith, 1954); many more must currently be under study. Perhaps in this material lies our best hope of an eventual understanding of the genetic basis for antigenic complexes. In the mouse, convenient phenotypic markers are available on one side of the region. It would be particularly desirable to find a useful marker on the other side, to permit definition of apparent recombinational events as they occur. In view of the ease and frequency of "recombination" for the *H-2* complex, however, we may still doubt the extent to which observations for this chromosomal region may prove to be typical of the antigen complexes encountered elsewhere, as in cattle and human blood-cell characteristics, for which "recombinations" have not been observed in spite of considerable opportunity for their observation. Also, this part of the ninth chromosome of the mouse has some queer characteristics, as witness the behavior of the dominant tail gene markers linked with the *H-2* region and studied in detail by Dunn (1957) and others. It is likely here, too, that independent and separable genic effects will need to be distinguished from intragenic and haptenic modifications, and all these and their interactions may be found to contribute to the diversity of effects observed for this chromosomal region. The game is likely to prove difficult and frustrating at times, but it will surely be "worth the candle."

Immunogenetics in its broader context comprises much more than the restricted subjects to which I have referred in this brief review. Serology has served as a productive handmaiden to various parts of the field of genetics, and it is likely to do so increasingly in the future. For example, the use of enzymes as antigens and the identification of antigenic materials related to enzymes have contributed in important ways to biochemical genetics and toward an eventual understanding of the genetic control of enzyme specificity and activity (Cohn and Torriani, 1953; Markert and Owen, 1954; Suskind, 1957). The recognition of tissue-specific antigens, of stage-specific antigens during development, and particularly the immunogenetics of histocompatability antigens for studies of cancer and of the transplantation of normal tissues has led to whole areas of important work (Tyler, 1957; Owen, 1959). The case of "ciliary" antigens of protozoa, in which the genotype controls sets of mutually exclusive potentialities whose realization can be affected by environmental modifications (Sonneborn, 1948; Beale, 1954; Nanney, 1957; Margolin, Loefer, and Owen, 1959), has contributed concepts important to an eventual understanding of mechanisms of differentiation in multicellular organisms. Serological classifications of many forms, particularly viruses and bacteria, and the use of serological markers for genetic studies of these organisms and of such material as vertebrate somatic cells *in vivo* (Cotterman, 1958; Atwood and Scheinberg, 1958) and *in vitro* (Pontecorvo, 1959); the remarkable changes in antigenic specificity of *Salmonella* associated with the establishment of phage infections (Uetake, Luria, and Burrows, 1958); phase variation in *Salmonella* and its genetic control (Lederberg and Iino, 1956); immunogenetic studies of *Drosophila* extracts, which are likely to become more definitive as techniques for the serology of complex mixtures are developed and applied (Barish and Fox, 1956); and the "immuno-

genetic" quality of self-incompatibility in plants (Lewis and Crowe, 1958)—all these diverse subjects and many more lie at least in part in the province of immunogenetics.

In its broadest sense, immunogenetics deals with the specificities of molecules, particularly macromolecules, and their inheritance and interactions. The contributions of immunogenetics to physiological genetics have been diverse and extensive, and we may expect even richer contributions in the future.

REFERENCES

ALLEN, S. L. 1955. Linkage relations of the genes histocompatibility–2 and fused tail, brachyury and kinky tail in mouse, as determined by tumor transplantation. Genetics *40*: 627–650.

ATWOOD, K. C., and S. L. SCHEINBERG. 1958. Somatic variation in human erythrocyte antigens. *In* Symposium on Genetic Approaches to Somatic Cell Variation. J. Cellul. Physiol. *52* (suppl. 1): 97–123.

BARISH, N., and A. S. FOX. 1956. Immunogenetic studies of pseudoallelism in *Drosophila melanogaster*. II. Antigenic effects of the vermilion pseudoalleles. Genetics *41*: 45–57.

BEALE, G. H. 1954. The Genetics of *Paramecium aurelia*. Cambridge: Cambridge University Press.

BILLINGHAM, R. E., L. BRENT, and P. B. MEDAWAR. 1953. "Actively acquired tolerance" of foreign cells. Nature, Lond. *172*: 603–606.

——— 1956. Quantitative studies on tissue transplantation immunity. III. Actively acquired tolerance. Philos. Tr. R. Soc., Lond., Ser. B. *239*: 357–414.

BRILES, W. E., W. H. MCGIBBON, and M. R. IRWIN. 1950. On multiple alleles affecting cellular antigens in the chicken. Genetics *35*: 633–652.

BURNET, F. M. 1956. Enzyme Antigen and Virus: A Study of Macromolecular Pattern in Action. Cambridge: Cambridge University Press.

——— 1959. The theories of antibody production. *In* A Symposium on Immunity and Virus Infection, ed., V. NAJJAR. New York: Wiley & Sons, Inc., pp. 1–17.

CEPPELLINI, R. 1952. Le varianti Rh. *In* R. CEPPELLINI, S. NASSO, and F. TEZILACICH, La malattia emolitica del neonato, p. 140. Milan: Istituto Sieroterapico Milanese, Serofino Belfanti.

——— 1959. Physiological genetics of erythrocyte antigens. *In* Symposium on Genetics in Medical Research, ed., J. LEDERBERG. To be published as supplements to Am. J. Human Genetics and J. Med. Educ.

COHEN, C. 1956. Occurrence of three red blood cell antigens in rabbit as the result of interaction of two genes. Science *123*: 935–936.

COHN, M., and A. M. TORRIANI. 1953. The relationships in biosynthesis of the β-galactosidase- and Pz-proteins in *Escherichia coli*. Biochim. biophys. acta, Amst. *10*: 280–289.

COONS, A. H. 1956. Histochemistry with labeled antibody. Internat. Rev. Cytol. *5*: 1–23.

COTTERMAN, C. W. 1958. Erythrocyte antigen mosaicism. *In* Symposium on Genetic Approaches to Somatic Cell Variation. J. Cellul. Physiol. *52* (suppl. 1): 69–95.

DUNN, L. C. 1957. Studies of the genetic variability in populations of wild house mice. II. Analysis of eight additional alleles at locus T. Genetics *42*: 299–311.

EHRLICH, P. 1900. On immunity with special reference to cell life. Proc. R. Soc. Ser. B. Biol. Sc., Lond. *66*: 424–448.

FISHER, R. 1953. Population genetics. Proc. R. Soc. Ser. B. Biol. Sc., Lond. *141*: 510–523.

GORER, P. A. 1956. Some recent work on tumor immunity. Advance. Cancer Res., *4*: 149–186.

HOECKER, G., S. COUNCE, and P. SMITH. 1954. The antigens determined by the *H–2* locus: A rhesus-like system in the mouse. Proc. Nat. Acad. Sc. U.S. *40*: 1040–1051.

INGRAM, V. M. 1957. Gene mutations in human haemoglobin: The chemical difference between normal and sickle cell haemoglobin. Nature, Lond. *180*: 326–328.

IRWIN, M. R., and L. J. COLE. 1936. Immunogenetic studies of species and of species hybrids in doves, and the separation of species-specific substances in the backcross. J. Exp. Zool. *73*: 85–108.

ITANO, H. A., and S. J. SINGER. 1958. On dissociation and recombination of human adult hemoglobins A, S, and C. Proc. Nat. Acad. Sc. U.S. *44*: 522–529.

JERNE, N. K. 1955. The natural-selection theory of antibody formation. Proc. Nat. Acad. Sc. U.S. *41*: 849–857.

KABAT, E. A. 1957. Size and heterogeneity of the combining sites on an antibody molecule. *In* Symposium on Antibodies: Their Production and Mechanism of Action. J. Cellul. Physiol. *50* (suppl. 1): 79–102.

LANDSTEINER, K., and J. VAN DER SCHEER. 1936. On cross reactions of immune sera to azoproteins. J. Exp. M. *63*: 325–339.

LEDERBERG, J. 1958. Summary comment. *In* Symposium on Genetic Approaches to Somatic Cell Variation. J. Cellul. Physiol. *52* (suppl. 1): 383–401.

LEDERBERG, J., and T. IINO. 1956. Phase variation in *Salmonella*. Genetics *41*: 743–757.

LEWIS, D., and L. K. CROWE. 1958. Unilateral interspecific incompatibility in flowering plants. Heredity *12*: 233–256.

MARGOLIN, P., J. B. LOEFER, and R. D. OWEN. 1959. Immobilizing antigens of *Tetrahymena pyriformis*. J. Protozool. (in press).

MARKERT, C. L. and R. D. OWEN. 1954. Immunogenetic studies of tyrosinase specificity. Genetics *39*: 818–835.

MORGAN, W. T. J., and W. M. WATKINS. 1956. The product of the human blood group *A* and *B* genes in individuals belonging to group *AB*. Nature, Lond. *177*: 521–522.

NANNEY, D. L. 1957. The role of the cytoplasm in heredity. *In* A Symposium on the Chemical Basis of Heredity, eds., W. D. MCELROY and H. B. GLASS, pp. 134–166. Baltimore: The Johns Hopkins Press.

OWEN, R. D. 1957. Immunological tolerance. Fed. Proc., Balt. *16*: 581–591.

——— 1959. Genetic aspects of tissue transplantation and tolerance. *In* Symposium on Genetics in Medical Research, ed., J. LEDERBERG. To be published as supplements to Am. J. Human Genetics and J. Med. Educ.

OWEN, R. D., C. STORMONT, and M. R. IRWIN. 1958. Studies on blood groups in the American bison (buffalo). Evolution *12*: 102–110.

PONTECORVO, G. 1959. The genetics of somatic cells. *In* Symposium on Genetics in Medical Research, ed., J. LEDERBERG. To be published as supplements to Am. J. Human Genetics and J. Med. Educ.

RACE, R. R., and R. SANGER. 1954. Blood Groups in Man. 2nd ed. Oxford: Blackwell Scientific Publications.

SCHEINBERG, S. L. 1956. Genetic studies of cellular antigens in the chicken. Genetics *41*: 834–844.

SCHWEET, R. S., and R. D. OWEN. 1957. Concepts of protein synthesis in relation to antibody formation. *In* Symposium on Antibodies: Their Production and Mechanism of Action. J. Cellul. Physiol. *50* (suppl. 1): 199–228.

SNELL, G. D., P. SMITH, and F. GABRIELSON. 1953. Analysis of the histocompatibility–2 locus in the mouse. J. Nat. Cancer Inst. *14*: 457–480.

SONNEBORN, T. M. 1948. The determination of hereditary antigenic differences in genically identical Paramecium cells. Proc. Nat. Acad. Sc. U.S. *34*: 413–418.

STORMONT, C. 1955. Linked genes, pseudoalleles and blood groups. Am. Natur. *89*: 105–116.

SUSKIND, S. R. 1957. Properties of a protein antigenically related to tryptophan synthetase in *Neurospora crassa*. J. Bact., Balt. *74*: 308–318.

TALMAGE, D. W. 1958. Mechanism of the antibody response. *In* Current Activities in Molecular Biology, ed., R. E. ZIRKLE. Chicago: University of Chicago Press.

TYLER, A. 1957. Immunological studies of early development. *In* The Beginnings of Embryonic Development, eds., A. TYLER, R. C. VON BORSTEL, and C. B. METZ, pp. 341–382. Washington, D.C. Publ. No. 48, Am. Ass. for the Advancement of Science.

UETAKE, H., S. E. LURIA, and J. W. BURROWS. 1958. Conversion of somatic antigens in *Salmonella* by phage infection leading to lysis or lysogeny. Virology *5*: 68–91.

WIENER, A. S., and I. B. WEXLER. 1956. The interpretation of blood group reactions, with special reference to the serology and genetics of the RH-HR types. *In* Novant' anni delle leggi mendeliane, ed. L. GEDDA, pp. 147–162. Rome: Instituto Gregorio Mendel.

CYTOPLASMIC INHERITANCE AND THE SEGREGATION OF PLASMAGENES

P. Michaelis[1]

In general, the existence of cytoplasmic inheritance is well recognized. But its significance for biology still seems to be undervalued. Above all, two methodological reasons are the cause of this depreciation. First, our investigation methods are not adequate for the recognition of all the present cytoplasmic differences. However, we shall not discuss this problem now (Michaelis, 1951). Secondly, the many various particles of the cytoplasm—plastids, chondriosomes, microsomes, nucleoprotein molecules—cannot be distinguished with our genetic methods. In many investigations this fact has not been sufficiently considered. The occurrence of a non-Mendelian inheritance, strictly speaking, demonstrates only an extrachromosomal inheritance because the behaviour of chromosomes alone causes Mendelian segregation. The occurrence of differences after reciprocal hybridization can logically only demonstrate genetic differences between female and male gametes. These gametes differ in the quantity of cytoplasm transmitted, but—so far as we know—do not differ greatly in the qualitative content of different cytoplasmic particles. Therefore, these two methods, by means of non-Mendelian inheritance or by reciprocally different hybrids, may well demonstrate genetic differences of the cytoplasm, but they cannot localize this difference, for instance, in the plastids, in other organelles, or in the optically clear cytoplasm itself. For these reasons I propose (Michaelis, 1949a) to designate the extranuclear sum of genetic units, demonstrable by these two methods, with the term "plasmon" and to include in this term the plastid inheritance, the inheritance by other cytoplasmic organelles, and the inheritance by hypothetical genetic units of the cytoplasm itself. This definition alone is in accord with our previous investigation methods.

A more detailed differentiation among the various cytoplasmic particles is only possible if specific differences in the behaviour of the single particles can be found during the multiplication of the particles, cells, or organisms and if the cytoplasmic inheritance follows the behaviour of such a particle in all details. A method for plasmon analysis must be sought, which corresponds to the analysis of chromosomes and nuclear genes during Mendelian segregation. That is to say, a segregation of cytoplasmic units must be analysed.

Where can such a plasmon segregation be found? I would like to remind you of the variegation of albomaculate plants, which is inherited in non-Mendelian manner only through the mother. Since the time of Baur such a variegation has been explained by a segregation and sorting out of genetically different "white" and "green" plastids.

Furthermore, plasmon segregation in some *Epilobium* species hybrids can be found. In these species hybrids, plasmons, normally absolutely constant during many generations, were inconstant through the influence of distinct nucleocytoplasmic combinations. On these plants, numerous alterations arise as leaf and

[1]Max-Planck-Institut für Züchtungsforschung, Köln-Vogelsang, Germany.

shoot sectors. The lateral buds arising in these leaf sectors produce various branches with different plasmotypes. Reciprocal crosses between the various branches of one single plant offer a maternal non-Mendelian inheritance over many generations and demonstrate that these different sectors and branches contain the same nuclear genes and differ only in their cytoplasm (Michaelis, 1949b, 1958b).

Characteristic of both examples of plasmon segregation is the intra-individual segregation. Cytoplasmic units can segregate not only during meiosis and fertilization at the end of the generations as do the chromosomal genes, but also during each cell division. This finding corresponds to the observation made with a microscope, that cytoplasmic particles can be distributed very irregularly during cell divisions. In the cytoplasm no mechanism is known which distributes the particles in an exact manner, similar to that by which mitosis distributes the chromosomes to the daughter cells.

The establishment of an intra-individual plasmon segregation leads to important methodological consequences especially in multicellular organisms. In unicellular organisms the segregation of plasmagenes can be analysed during the cell divisions of a clone, as Sonneborn has done in *Paramecium*. In multicellular organisms not only must the sexual progenies of a heteroplasmonic individual be investigated, but also the intra-individual patterns especially, which arise during the somatic cell divisions, must be analysed. The following report will be restricted to investigations of the multicellular plant *Epilobium*.

In order to acquaint ourselves with the interrelations between plasmon segregation and the origin of intra-individual patterns, the plastid segregation was investigated at first, as a model of plasmon segregation, because here the behavior of plastids can be followed up microscopically. The necessary plant material was produced in mutation experiments with radioactive isotopes. The spontaneous rate of variegated plants is very low, but was increased in these experiments (Table I).

TABLE I

RATE OF ALBOMACULATE MUTANTS OF *Epilobium*

		Number of cultivated plants	Percentage of albomaculate plants
Non-treated plants (control)		68.699	0.08
Treatment with S^{35}	<0.6 mc.	4.626	0.13
	0.12–0.15 mc.	2.958	0.27
	0.23–0.47 mc.	3.252	0.77

In Table I an increase of frequency of variegated plants with maternal inheritance (from 0.08 per cent to the tenfold 0.77 per cent) can be seen with an increase of dose. In these experiments (Michaelis, 1958c), besides nuclear gene mutations, 172 albomaculate plants were obtained. In all plants where the variegation reached the flowers, the variegation was inherited only through the mother in a non-Mendelian manner.

If all the different intra-individual mutants are examined, specific peculiarities of the patterns become apparent. The coincidence of the pattern areas with the areas of developmentally caused cell descendants is a significant property of all genetically induced patterns. With spots caused by injuries or infections, and with physiological patterns, such a coincidence is the exception.

Among the variegated plants with cytoplasmic inheritance two main groups with different patterns can be distinguished. In the larger group the spots of variegation

are distributed at random over leaves and plants. In my opinion such a distribution could only arise if the determining genetic units were distributed at random during the cell divisions. Such a distribution can be established by several methods. On leaves, in which the cells divided in all parts with the same frequency, the number of spots can be counted on the different parts of the leaves or the white areas can be planimetrically determined. A more exact method would be the comparison of two patterns which arise from sister cells of one cell division. Here we must briefly discuss the development of *Epilobium* leaves (Michaelis, 1957b). Figure 1 illustrates a scheme of the first cell divisions in a leaf beside a variegated leaf, on which

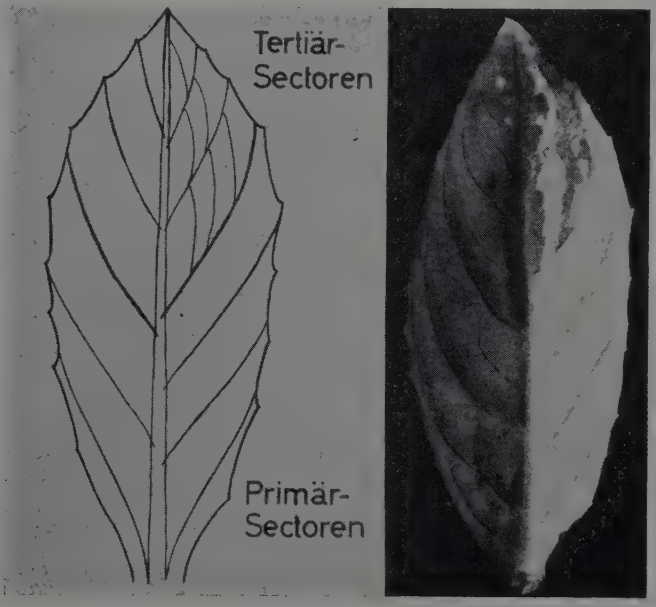


FIGURE 1

these divisions were perceptible by the position of patterns. First, in each lamina half three or four cell divisions parallel to the lateral veins divide the lamina in several primary sectors. The first of these divisions divides each half leaf into a basal and a top cell. On the leaf on the right side the basal cell produced a white cell descendant, the top cell was heteroplasmonic and its cell descendants segregate further. After secondary divisions parallel to the leaf surface, some cell divisions parallel to the main vein divide the primary sectors into tertiary sectors. Those tertiary sectors can be seen on the right top half of the leaf. For the exact establishment of random distribution, the frequency with which white primary sectors arise on the top or base of the leaf can be determined, or the number of white tertiary sectors at the main nerve or at the margin of the leaves can be counted. Table II reproduces some determinations.

On 7 of 128 variegated plants studied, an asymmetrical distribution of spots was found. In the example shown in Table IIB most spots lie on the top or margin of the leaves. I assume that in this plant the greater part of the mutated cytoplasmic units wandered into the top cell during the first cell divisions, and into the marginal cells during the tertiary cell-division period. Unfortunately, it was not possible to investigate the behavior of plastids microscopically because mutated plastids lose

TABLE II

DISTRIBUTION OF CYTOPLASMIC UNITS

A. RANDOM DISTRIBUTION (121 FROM 128 VARIEGATED PLANTS)

Half of leaves	Planimetric determinations (white area as percentage of total leaf area): Plant 1954.1111 (19 leaves)	Planimetric determinations (white area as percentage of total leaf area): Plant 1956.241.44 (12 leaves)	Sectors	Developmental determinations (number of white sectors): Plant 1956.1323.82	Developmental determinations (number of white sectors): Total of 12 plants
Basal	36.8%	52.5%	Basal primary	29	171
Top	37.8%	56.6%	Top primary	28	179
Central	35.4%	51.5%	Central tertiary	36	118
Marginal	39.2%	57.6%	Marginal tertiary	35	123

B. UNEQUAL DISTRIBUTION (PLANT 1955. 4411. 27)

Position of white spots on leaves	Leaves of main shoot: 1955	Leaves of main shoot: 1956	Leaves of lateral branches	Leaves of F_2 plants	Total
Top or margin	21	27	30	11	89
Center or base	2	10	5	5	22

their chlorophyll only in the adult cell. In the dividing cell normal and mutated plastids cannot be distinguished.

It is important that in variegated plants a random distribution of cytoplasmic units is found most frequently. In these cases the laws of plastid segregation can be derived by comparison of the patterns with model experiments (Michaelis, 1955a) or by mathematical calculations (Michaelis, 1955b). In model experiments a number of white and green balls are put into a box, corresponding, for example, to the number of white and green plastids in the cell before the cell division. After a random halving of the balls not all ratios of white or green balls have the same frequency (Figure 2). In the upper part of the figure eleven curves are drawn, which demonstrate the frequencies of ratios with a total of fifty balls when the ratio between white and green balls was 4 : 46 before the division, 8 : 42, 12 : 38, and so on. After the cell division the same ratio as before the division occurs most frequently. The frequency of other ratios decreases to the same extent as these differ from the ratio before the division. The curves show furthermore that the segregation curves with different starting ratios are very similar, as the lower left side of Figure 2 demonstrates. Here the curves for different starting ratios are drawn one upon another with the starting ratio as zero-point. The curve on the right side will be discussed later.

These results of the model experiments and mathematical calculations have two important consequences for cytoplasmic inheritance. With random segregation, it is impossible that cells possessing only mutated units—that is, homoplasmonic mutated cells—arise in a few cell divisions from cells in which only one of the numerous units is altered by mutation. During a longer period of cell divisions only cells arise which contain mutated and normal units side by side in one cell. These cells, termed by Correns "mixed cells," characterize all cases of plastid inheritance, in which plastogenes determine the appearance of the plastids directly and in which interactions between plastids and other cell organelles are lacking (Michaelis,

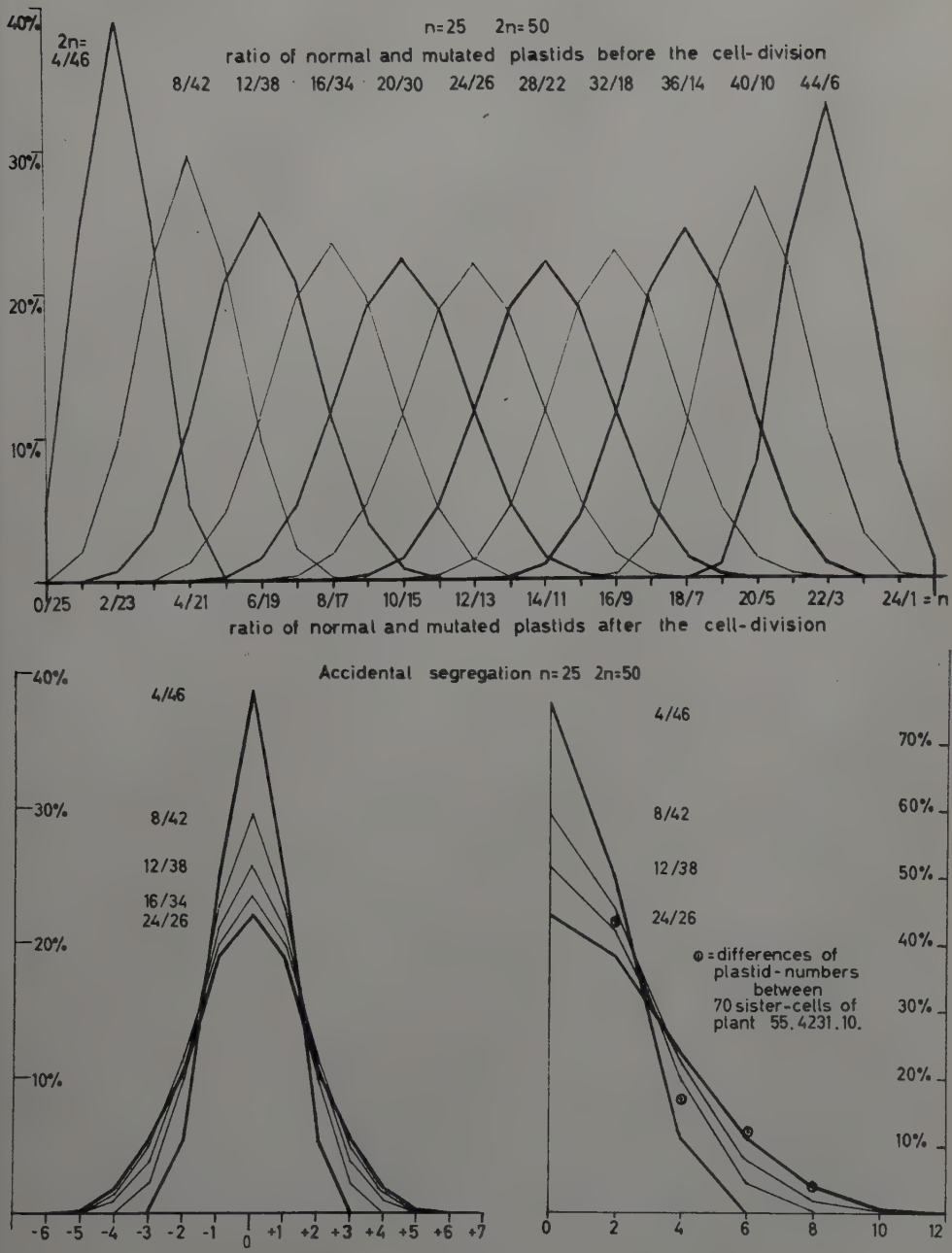

n=25 2n=50
ratio of normal and mutated plastids before the cell-division
2n= 4/46
8/42 12/38 16/34 20/30 24/26 28/22 32/18 36/14 40/10 44/6
40%
30%
20%
10%
0/25 2/23 4/21 6/19 8/17 10/15 12/13 14/11 16/9 18/7 20/5 22/3 24/1 = n
ratio of normal and mutated plastids after the cell-division
Accidental segregation n=25 2n=50
4/46
8/42
12/38
16/34
24/26
-6 -5 -4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7
4/46
8/42
12/38
24/26
⊙ = differences of plastid-numbers between 70 sister-cells of plant 55.4231.10.
70%
60%
50%
40%
30%
20%
10%
0 2 4 6 8 10 12

FIGURE 2

1957a). A cytological investigation of 172 albomaculate plants obtained in mutation experiments showed mixed cells in fifty plants. In thirty albomaculate plants mixed cells were definitely lacking. These albomaculate plants will be discussed later on. In the remaining ninety-two plants the existence of mixed cells could not be established with certainty because the differences between normal and mutated plastids were too small.

A second consequence of model experiments and calculations is the facilitation of diagnosis of mixed cells proper and the distinction of plastid mutants from physiologically modified plastids. In model experiments great differences between starting ratios and segregation ratios are rare. It follows that in sister cells arising from one dividing mother cell similar mixed ratios must occur. Figure 3 reproduces a drawing of palisade cells in optical cross-section. In the investigated plant the cells lie

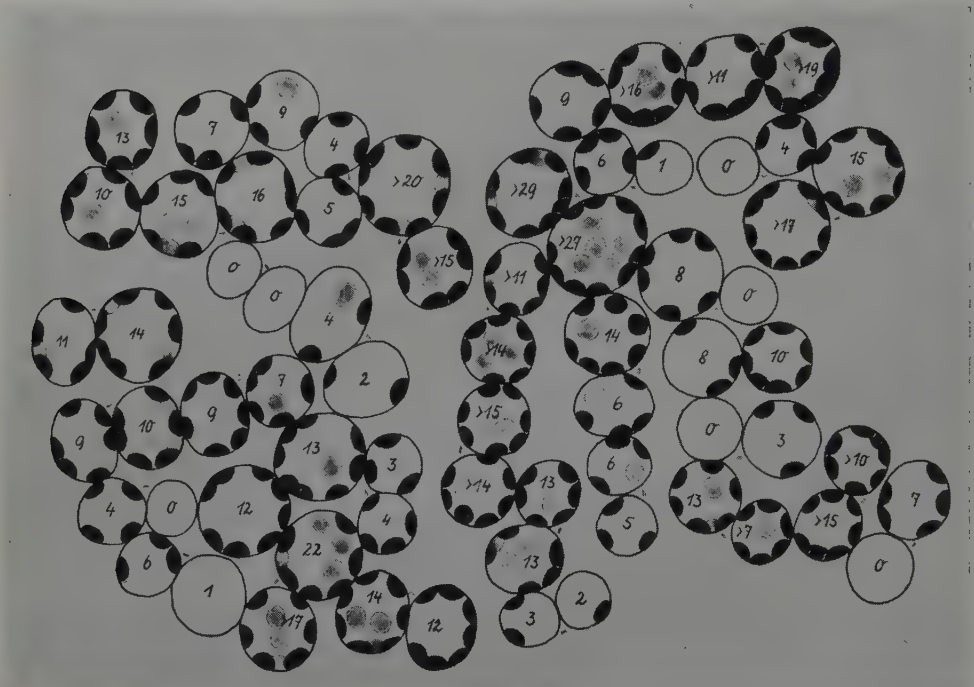


FIGURE 3

exceptionally loose, as a result of absence of the last cell divisions, so that the developmental connections could be reconstructed by the position of cells and intercellular spaces. Sister cells are connected by lines in Figure 3. The numbers show how many normal plastids there are in mixed cells with degenerating plastids. At first sight similar values are found in sister cells. The curves on the lower right side of Figure 2 are based upon the same values as the other curves, but here the curves demonstrate the theoretical expectations of differences between sister cells. The circled points offer microscopic countings of plastid differences in the palisade cells of *Epilobium* leaves. Theoretically and experimentally determined values correspond to a high degree. These and other agreements between model experiments, mathematical calculations, and behavior of plastids demonstrate that the variegation of the investigated plants with mixed cells is really caused by random

segregation of genetically different plastids. It should be possible to employ the method of analysis of intra-individual patterns, well tested in plastid inheritance, in cases in which the genetic units cannot be seen microscopically.

As already mentioned, approximately one-third of the experimentally induced variegated plants possessed no mixed cells. It was characteristic of these plants that frequently single cells or small cell groups were found containing only abnormal

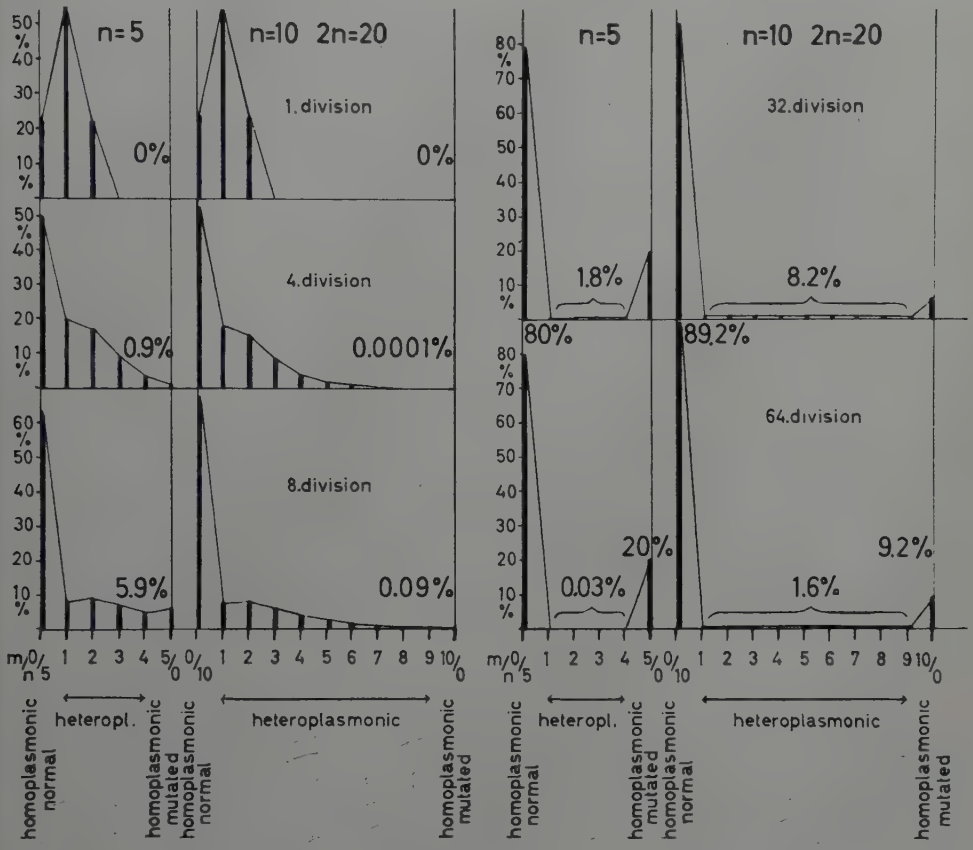


FIGURE 4

plastids in the midst of normal cells without any mutated plastids. Such an occurrence is impossible with a random segregation of genetically different plastids. It is probable that in these cases the plastid development depends not only upon the plastogenes of each plastid, but also upon substances diffusing through the whole cell and influencing all plastids of one cell in a similar manner. Without special investigations the origin of these substances remains unknown. But indications of their origin can be drawn from the course of segregation because the velocity of sorting-out of homoplasmonic cells depends upon the number of genetic units per cell.

As an example, Figure 4 demonstrates the theoretical course of segregation (Michaelis, 1955a, b) during many cell divisions after a mutation. For comparison the curves are drawn side by side for $n = 5$ and $n = 10$ units per cell, respectively, after the division, or $2n = 10$ and $2n = 20$ units per cell before the cell division.

During the first cell divisions homoplasmonic normal and heteroplasmonic cells can arise, but no homoplasmonically mutated cells. Only after some cell divisions can homoplasmonically mutated cells be expected. After four cell divisions 0.9 per cent homoplasmonically mutated cells may be expected at five units per cell. At ten units per cell their frequency lies at 0.0001 per cent. After eight cell divisions at five units per cell 5.9 per cent homoplasmonically mutated cells can be expected, and at ten units per cell only 0.09 per cent. In general, during the further cell divisions the number of homoplasmonic cells increases, the number of heteroplasmonic cells decreases. At five units per cell the heteroplasmonic cells practically disappear after fifty cell divisions, at ten units per cell more than a hundred cell divisions are necessary for a complete sorting-out. Furthermore, the calculations show that after the complete sorting-out the relation between homoplasmonically mutated cells and homoplasmonically normal cells is the same as the relation of mutated and normal units in the cell shortly after the mutation; for example at ten units, the proportion is 1 : 9, at five units 1 : 4. Figure 5 demonstrates the velocity

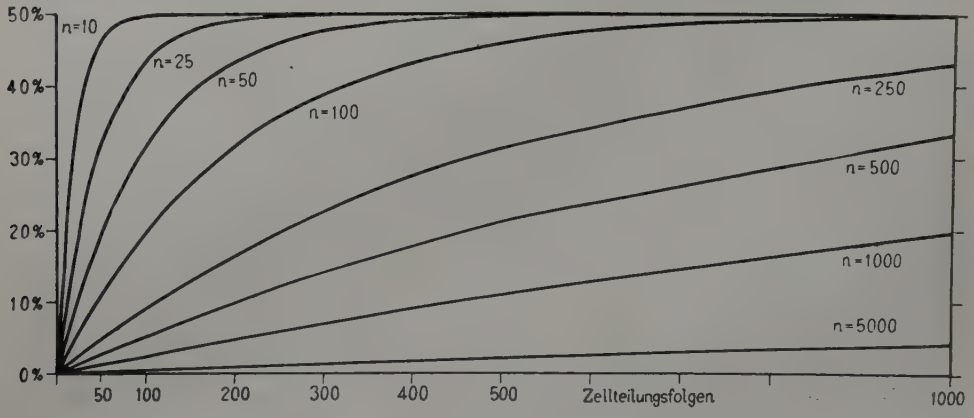


FIGURE 5

of sorting-out in percentage in relation to the number of units per cell ($n = 10$ up to $n = 5{,}000$) and its dependence on the number of cell divisions. The values show that with low numbers of segregating units the cell divisions of one individual suffice for a complete sorting-out in most plant species. Therefore mutations of cell organelles such as plastids and microsomes can be found. With high numbers of units —probable for the units of the cytoplasm itself—a quick sorting-out within one or several generations seems to be impossible as long as the segregation is random. The results of these calculations should enable us to localize cytoplasmic factors in distinct cytoplasmic particles since the number of plastids, chondriosomes, microsomes, and protein macromolecules is very different and specific for the various cells. It should be possible to distinguish plastid mutations from microsome or cytoplasm mutations if we can determine the point of mutation and the number of cell divisions between mutation and sorting-out.

The location of a spontaneous or induced mutation must be sought in the neighbourhood nearest the point on which all the descendants of a heteroplasmonic cell converge (Michaelis, 1957b). The place of sorting-out can be statistically established by counting the single white cells or by determining the number of cell divisions within white spots. The determination of the number of cell divisions which bring about white spots or which pass between mutation and sorting-out is especially

difficult and requires extensive investigations of the development of the object. In most cases the conventional knowledge of development does not suffice for the new method of localization. Hitherto it has only been possible to test the new method and to determine the necessary values and the sources of error in some favourable cases.

In one leaf of a variegated plant without mixed cells a back mutation was found in which cytoplasmic units producing white plastids mutated to green. The mutation arose during the first cell divisions of the leaf (Michaelis, 1957b). The whole segregation from mutation to sorting-out occurred during the development of this single leaf. Here the number of cell divisions necessary for the development of the leaf and of the white spots could be determined at least in order of magnitude. In

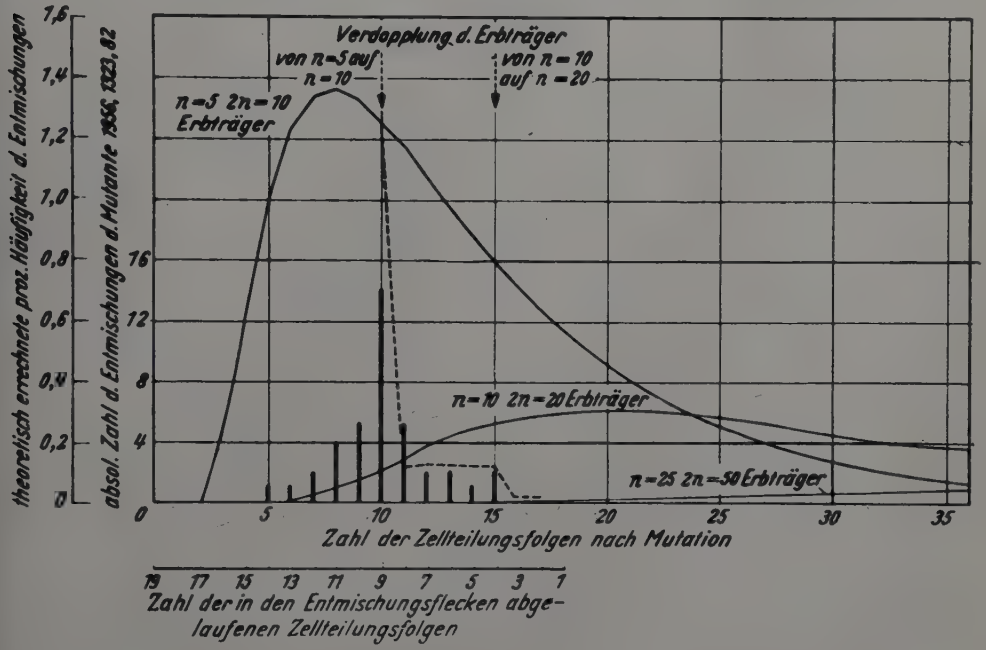


FIGURE 6

Figure 6 the heights of the vertical black columns represent the number of sortings-out in the different cell divisions, the number of which is marked on the abcissa. The maximum of sorting-out occurs at approximately ten cell divisions. The curves of the figure reproduce the theoretical calculations: the highest curve demonstrates the values of sorting-out at $n = 5$ or $2n = 10$ units per cell, the intermediate curve shows the values for ten or twenty units per cell, and the lowest curve on the right side below reproduces the values for $n = 25$ or $2n = 50$ units per cell. The values for higher numbers can be disregarded. The values of actual sorting-out lie within the range of the curves for $2n = 10$ and 20. Only the plastids occur in these low numbers in the cells. The real number of plastids in the meristematic cells lies between five and twenty with an average value of twelve. These numbers are doubled during the last cell divisions of the leaf to about twenty-five, and further to approximately fifty. The number of other cell particles is much higher. The number of microsomes amounts to more than two hundred. There can be no doubt that in this case the variegation is caused by a plastid mutation.

If mixed cells with normal and mutated plastids side by side cannot be found,

interrelations between the two types of plastids must be assumed. Probably substances arise which diffuse through the cell and influence all plastids in a similar way. This hypothesis could be confirmed by cytological investigations in some other variegated plants. Here, the theoretically supposed mixed cells were found in young leaves, but in older leaves the genetically normal plastids of mixed cells degenerate under the influence of the mutated plastids. The heteroplasmonic cells become white. In some other variegated plants the heteroplasmonic cells are green. Both cases can be distinguished by a microscopic investigation of cell patterns. If the heteroplasmonic cells are green, the heteroplasmonic green cell descendants split off white cells and within green areas little white cell groups or single white cells arise. If the white cells are heteroplasmonic, single green cells lie within white areas (Michaelis, 1957b).

In other cases of variegation (Michaelis, 1955a, b) without mixed cells, the sorting-out of cytoplasmic units takes many more cell divisions than would be expected with plastid inheritance. The sorting-out does not occur during the approximately twenty to thirty cell divisions of one leaf and the first white spots do not arise until the shoot develops several leaves. These investigations were made on the F_1 seedlings of a variegated plant without mixed cells. The number and course of cell divisions during embryo development are well known from embryological investigations (Bartels, 1956), and it can be calculated how frequently white patterns, for example white half-cotyledons, must arise in the seedlings with different numbers of segregating units per cell. In Figure 7 the curve reproduces the theoretical calculations for the occurrence of white half-cotyledons as it is dependent upon the number of segregating units per cell. The vertical column demonstrates the theoretical expectation for a plastid inheritance, the horizontal area shows the experimental results. With plastid inheritance, 7 to 21 white half-cotyledons must be expected among 124 half-cotyledons of the experiment. But no white halves were found. The cotyledons possess only small spots, and larger white

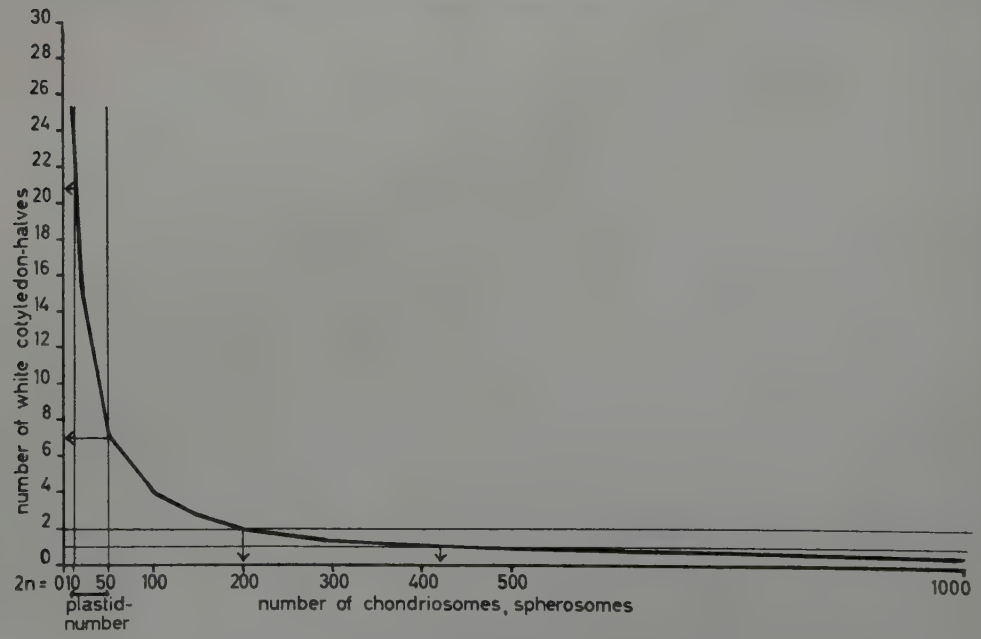


FIGURE 7

areas arise only on the leaves above the cotyledons. It must be assumed that the number of segregating units is considerably larger than the number of plastids, probably about the number of chondriosomes or spherosomes. Evidently this variegation does not arise owing to segregation of plastids. Probably albomaculate plants can also be produced by a segregation of other cytoplasmic particles, which influence the physiology of plastids.

These considerations may demonstrate that an analysis of the cytoplasmic inheritance is possible, if the hybridization method can be complemented by an analysis of intra-individual patterns. This new method encounters some difficulties as long as our knowledge of the development of the objects is as deficient as it is at present. As soon as the number and the sequence of cell divisions are known in the different objects, we can hope to obtain more exact values. Furthermore, the number of cytoplasmic particles and their variation during the ontogeny must be determined. For the present, only estimations of the order of magnitude and investigations of especially favourable cases are feasible. But I believe that this field of investigation is very important. The significance of plasmon does not lie only in the field of inheritance from generation to generation. The intra-individual segregation of plasmagenes also demonstrates the significance of plasmon for the development of the individuals and for the determination of their tissues. The importance of this role of cytoplasmic inheritance has hitherto not been understood because of the exclusive use of the hybridization method. The investigation of intra-individual segregation of plasmagenes will place the significance of cytoplasmic inheritance in its proper light.

REFERENCES

BARTELS, F. 1956. Zur Entwicklung der Keimpflanze von Epilobium hirsutum I. Die im Proembryo ablaufenden Zellteilungen bis zum beginnenden "herzförmigen Embryo." Flora *144*: 105–120.

MICHAELIS, P. 1949a. Prinzipielles und Problematisches zur Plasmavererbung. Biol. Zbl. *68*: 173–195. Vortr. Deut. bot. Ges. Berlin 1944.

——— 1949b. Ueber Abänderungen des plasmatischen Erbgutes. Zschr. indukt. Abstamm. *83*: 36–85.

——— 1951. Ueber die allgemeine Verbreitung plasmatischer Erbträger und ihre Bedeutung für die Entwicklungsphysiologie. Ber. Deut. bot. Ges. *64*: 196–207. Vortr. Deut. bot. Ges. Berlin.

——— 1953. Das labile Gen Pallidovariabile von Epilobium, seine Manifestation und Entstehung in verschiedenen Plasmonabänderungen. Zschr. indukt. Abstamm. *85*: 282–296.

——— 1954. Cytoplasmic inheritance in Epilobium and its theoretical significance. Advance Genet. *6*: 287–401.

——— 1955a. Modellversuche zur Plastiden- und Plasmavererbung. Züchter *25*: 209–221.

——— 1955b. Ueber Gesetzmässigkeiten der Plasmon-Umkombination und über eine Methode zur Trennung einer Plastiden-, Chondriosomen-, resp. Sphaerosomen-, (Mikrosomen)- und einer Zytoplasmavererbung. Cytologia *20*: 315–338.

——— 1957a. Ueber die Vererbung von Plastidenmerkmalen. Protoplasma. Lpz. *48*: 403–418.

——— 1957b. Genetische, entwicklungsgeschichtliche und cytologische Untersuchungen zur Plasmavererbung. II. Ueber eine Plastidenmutation mit intracellularer Wechselwirkung der Plastiden, zugleich ein Beitrag zur Methodik der Plasmonanalyse und zur Entwicklungsgeschichte von Epilobium. Planta *50*: 60–106.

——— 1958a. Plasma-Vererbung. Handb. Pflanzenzücht. Berlin, 2. Aufl., *1*: 140–175.

——— 1958b. Ueber die Bedeutung des plasmatischen Erbgutes für die Mannigfaltigkeit der Gattung Epilobium. Biol. Zbl. *77*: 165–196.

——— 1958c. Untersuchungen zur Mutation plasmatischer Erbträger, besonders der Plastiden. Planta *51*: 600–634, 722–756.

——— 1958d. The genetical interactions between nucleus and cytoplasm in Epilobium. Exp. Cell Res. Suppl. *6*: 236–257.

DARWIN CENTENNIAL SYMPOSIUM

GENETICS IN EVOLUTION

1. L. L. Cavalli-Sforza. Some Data on the Genetic Structure of Human Populations
2. Bruce Wallace. The Role of Heterozygosity in *Drosophila* Populations
3. Danko Brncic. The Mesophragmatica Group as an Example of the Speciation Phenomena in *Drosophila*
4. Jens Clausen. Gene Systems Regulating Characters of Ecological Races and Subspecies
5. Stanley G. Smith. The Cytogenetic Basis of Speciation in Coleoptera

SOME DATA ON THE GENETIC STRUCTURE OF HUMAN POPULATIONS

L. L. Cavalli-Sforza[1]

IT HAS BEEN for some time a commonplace of human genetics to consider man a poor object of genetic study in spite of the fact that since geneticists themselves belong to this species a study of man becomes as desirable or more desirable than that of any other species. But in recent years it has been increasingly recognized that man can be a good, if not optimal, organism for certain types of genetic problems, in spite of the disadvantages caused by the fact that the life span of the research worker is no longer than the generation time of the species under analysis. There are, of course, problems for which man has been a favorite object of study in fields other than that of genetics, but which supply information interesting from a genetic point of view. They range from biochemistry and medicine to history. One of them is the study of the genetic structure of populations; demography of the human species is more developed than that of any other species and can offer real help to an adequate description of population structure and an analysis of some of its problems.

Population structure can perhaps be defined best in a negative way, i.e. as the ensemble of known factors governing changes in gene or genotype frequencies other than mutation or selection. It represents the limits set to the action of these two primary evolutionary forces by the fact that any population is not infinite in size, and only approximates the simplifying conditions of random mating, which is the basis of the simplest evolutionary predictions.

In the theory of population structure, which has been presented by Wright (1943, 1946, 1951), we have the choice between two models which represent two somewhat extreme population structures. In one (the island model) a number of small population units or clusters, each of effective size N, all exchange genes with any other cluster, m being the effective proportion of the gametes of a cluster which is replaced in each generation by immigrants. Here we are required to estimate the two parameters N and m in order to define our population.

According to the alternative model (continuous distribution), the population is not grouped in clusters but is diffuse and mobile over an area or along a line (a strip of land or water). It is then considered as a population of units moving at random with given mobility and density; and essentially one parameter is required to describe it from a genetic point of view. Such a parameter has been called "neighborhood," and is defined mathematically as the inverse of the probability of self-fertilization of an individual. In the linear case, the neighborhood is calculated from $N = 2\sqrt{(\pi\sigma)d}$ where d is population density and σ is the standard deviation of the distance between birth places of parent and offspring. Individual mobility is assumed to operate according to the laws of normal diffusion. A range such as that occupied by a number of individuals equal to N includes 92.4 per cent of the actual parents of the individuals at the center. In the area model the neighborhood is $N = 4\pi\sigma^2 d$, and is equivalent to the number of individuals in a circle of radius 2σ. It includes 86.5 per cent of the parents of the individuals at the center.

[1]Laboratorio di Genetica (Istituto di Zoologia), Università di Parma, Parma, Italy.

If we want to make a decision in favor of one of these models in order to describe a real population, we must somehow conduct an analysis of the tendency to a formation of clusters in the population under study and of the geography and stability of these clusters.

When we think of a human population, one distinction of importance is that between its urban and rural parts. For urban populations the continuous model may seem appropriate inside the town, not forgetting, however, all the intricacies resulting from social stratification. But for some rural populations it seems that the clustering in villages is sufficiently pronounced that it has to be taken into account.

I have had an opportunity, in the last few years, to investigate a human population in an area of northern Italy, the Parma Diocese. I shall draw largely from the results attained in this investigation for the purpose of illustrating the topics under discussion. The work has proceeded along several interconnected lines, and would have been impossible without the help of a number of collaborators. The results, of which only a very preliminary and partial analysis has been published (Cavalli-Sforza, 1956), will appear in full in due time. Demographic records kept independently and in somewhat different form, by both governmental and religious authorities, are available in some cases for more than three centuries. The Parma Diocese includes about three-quarters of the Parma province with a total population of about 300,000. It covers a wide variety of physical conditions from mountains, which are scarcely inhabited, to plains with a high population density. Except for the immediate surroundings of towns where industry is prevalent, its economy is essentially agricultural. Changes in social and hygienic conditions characteristic of our contemporary world seem to have affected breeding patterns only in the last few decades and to a moderate extent.

Village Size

An idea of the spatial distribution of this essentially rural population and its tendency to cluster can be obtained from an analysis of the distribution of the sizes of "villages" (Fig. 1, based on the population census of 1951). We can distinguish at least three levels in the clusters of houses. The first, the smallest identifiable physical clusters, are usually not endowed with community centres ("nuclei abitati"). A fairly large proportion (more than one-quarter) of the total population lives in isolated houses, while the rest (about one-half, excluding the town) live in the "nuclei e centri abitati," which have a median size of forty-five people. All these scattered houses and small clusters, however, are attached to centers, called "frazioni," which do not have administrative independence but which form religious or social centers (second level). The smallest administrative units (third level) are the "comuni," with a median number of inhabitants approximately one hundred times that of a "nucleo abitato" and ten times that of a "frazione."

The religious unit, the parish, is almost identical with the "frazione." It is thus a relatively small unit; advantageously so from our point of view, because religious records, which take the parish as the geographical unit, are as a consequence more detailed from the point of view of spatial relations than are governmental records, which take the "comuni" as the smallest geographical unit. The social importance of the parish is such that it forms a "village," although in some cases one would, of course, like to be able to break it down further.

If we take the parish as the cluster, then enumeration of the people living in it and multiplication by simple factors might supply a direct estimate of "effective

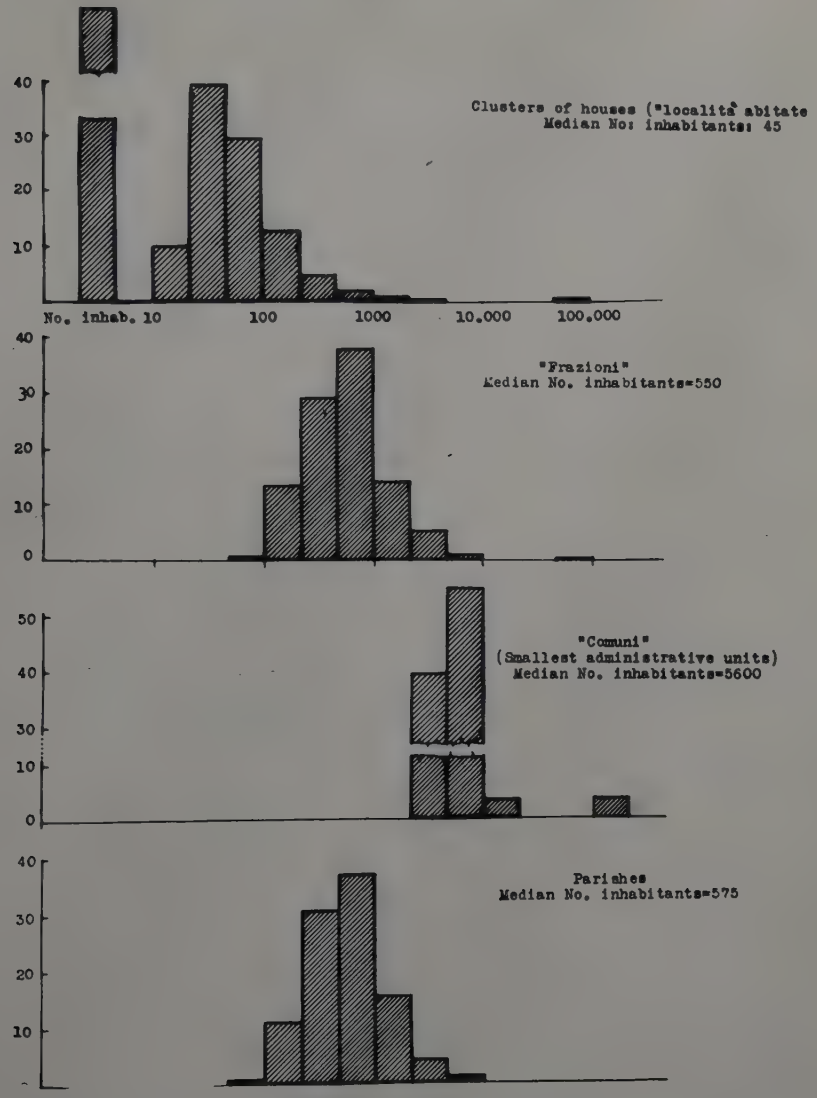


FIGURE 1. Distribution of village sizes in the Parma Diocese (1951 census).

size." It is the easiest to obtain and naturally the least accurate because, however strong the *a priori* considerations are for considering the parish as the population unit, its choice for this purpose is clearly arbitrary. Moreover, a parish is often socially heterogeneous and social heterogeneity will tend to reduce the effective size calculated from the parish total. In a similar calculation Böök (1956) had evidence that the "effective units" calculated from Dahlberg's formula were smaller than a parish. Swedish parishes are, however, probably larger on an average than Italian ones and are more similar to our "comuni."

If, as a first approximation, we take the parish as the cluster or population subgroup for use in Wright's island model, then the calculation of "effective size" N

demands only the knowledge of which fraction of the population is genetically active, or more exactly the number of gametes used per generation. This can be obtained from the number of fertile marriages per generation multiplied by four, or by similar related methods (Böök, 1956; Glass *et al.*, 1952). When only census data are available, a convenient approximation is to calculate the number of marriages per generation by multiplying village size $\times$ generation time in years $\times$ 0.007. This figure is derived from the ratio of marriages per year to number of inhabitants, and is a demographical constant which has a remarkable stability, even over long periods, but may vary somewhat from country to country.

In the present instance, N turns out to be equal to about 88 per cent of the village size.

Migration

Estimation of migration can be easily made from parish data because marriage books, at least those available to us, indicate the parish of residence and sometimes of birth of the spouse. Actually, one would like to know the birth places of parent and offspring to be able to cover migration over a full generation cycle, and this can in some instances be done. In the population which I have analyzed a substantial part of the total amount of migration taking place over one generation is covered by using the distance between the residences of the spouses.

More than 99 per cent of the marriages are performed in the Catholic Church and are, therefore, recorded in the parish books. According to the local rules, marriage almost always takes place in the bride's parish. After marriage the couple usually reside either in the bridegroom's parish or in the bride's parish, a little oftener in the former.

One can analyze migration by the fraction of marriages where both partners are from the same parish; various coefficients (for example, hexogamy coefficient, Freire-Maia, 1952, 1957) are simple functions of this fraction. Here we shall refer to E and "endogamy" as the fraction or percentage of marriages in which both spouses reside in the same parish. Or, alternatively, one can examine the whole distribution of distances between the residences of the spouses, as has been done repeatedly by several demographers, sociologists, and geneticists (Sutter and Tran-Ngoc-Toan, Schwidetzky, quoted by Freire-Maia, 1957a; Bossard *et al.*, 1932).

It should be noted that the two modes of analysis may be completely unified, and in a very easy way if the whole distribution can be expressed by a single parameter. Actually, it turns out that we are close to this ideal.

Let us, therefore, examine first the distribution of distances. Our present data represent a stratified sample of twenty-one out of the ninety parishes of the Parma valley (to be described later), in which the marriages contracted from 1800 to 1950 were analyzed from this point of view.

The observed distribution is given in Figure 2. Classes were chosen according to a quadratic proportion of distances, to account for rapid falling-off of frequencies with distances. The theoretical curve for normal diffusion, corresponding to the equation:

$$f(x, y)\ \frac{1}{\pi v}\ e^{-(x^2+y^2)/v}$$

where v is the mean square distance from the origin, x and y the distances in rectangular co-ordinates from the origin, is given in Figure 4, in a form suitable for

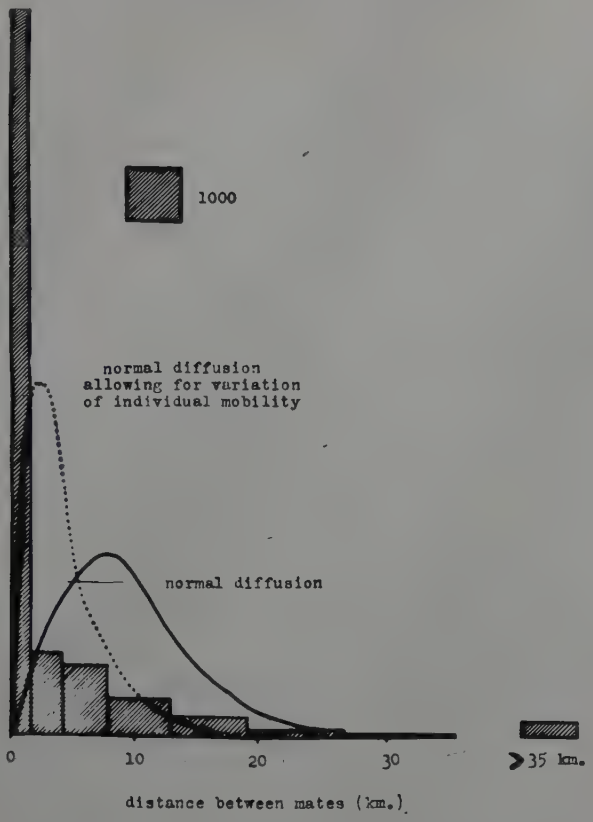


FIGURE 2. Distribution of 8,665 marriages according to distance of place of residence between mates.

graphical representation, that is, as the distribution of distances from the origin r ($r = \sqrt{(x^2 + y^2)}$

$$f(r) = \frac{2r}{v} e^{-r^2/v}$$

where v is the mean value of r^2. Clearly this theoretical curve is very far from describing matrimonial migration. In statistical terminology one can say that the actual distribution deviates from the normal in being highly leptokurtic.

The leptokurtic aspect of distributions of biological diffusion has been established before, and also for other organisms, for example, insects (Bateman, 1950). An interesting suggestion by Skellam (1951) is pertinent here. This author has considered the effect of variation in individual mobility or migratory tendencies. The result is a leptokurtic distribution which, by assuming a certain distribution of individual mobility (more precisely a gamma distribution of $1/v$), turns out to give a good fit, for example, for the wanderings of millipedes. It is likely that human individuals differ one from the other even more than millipedes in the degree of their sedentary or nomadic tendencies, in their initiative, allegiance to or rejection of tradition, taste for the new, etc., and it was anticipated that some such variation would be found.

However, even assuming an extreme variability of individual mobility (such as a J-like, or almost J-like, distribution), according to Skellam's model we are not yet able to cope with the extreme leptokurtosis of the data (Fig. 2, dotted curve).

This is not too surprising, considering how far the actual situation is from that of ordinary diffusion. If the standard model of diffusion were correct, an individual would be assumed to wander like a molecule, and every movement would start from the place where the individual arrived last without any memory of previous whereabouts. In reality, everyone goes home almost every night and explores a limited territory during his daily activities. Usually he has a chance of meeting his or her future mate in places which are most of the time at a distance from home of one day's walk or travel. He has, therefore, a good chance of exploring the immediate neighborhood fairly well. Diffusion would come only if he changed residence repeatedly, but this is an infrequent event in rural populations.

In the lack of a good model to represent this type of migration, we may look at least for an adequate description of the data. Previous experience with insects (Bateman, 1950) and partially with man (Sutter and Tran-Ngoc-Toan, 1957; Fraccaro, 1958) showed a good fit of an empirical function

$$F = ce^{-k\sqrt{r}}.$$

The constant c is equal to $k^2/2$ and this function depends on one parameter only. Sutter and Tran-Ngoc-Toan (1957) have found that some distributions are to be fitted instead with the function e^{-kr}.

If we try to apply this empirical formula to our data, especially if we try to fit the data from individual parishes, we have to take account somehow of the vagaries of the geographical distribution of the population, which greatly distorts the probability of marriage as a function of distance alone ($f(r)$). Instead of estimating this we can take the number of marriages at a given distance divided by the number of people living at that distance,[2] and then obtain a probability $p(r)$ of marrying any given individual at a given distance. In other words while $f(r)$ expresses a probability per kilometer, $p(r)$ expresses a probability per kilometer person. If, however, we prefer the former type of probability, $f(r)$, we can account for the vagaries of geographical distribution by dividing $f(r)$ by population density at distance r. The two values $f(r)$ and $p(r)$ are easily transformable one into the other, but we should keep in mind that in a homogeneously distributed population the number of inhabitants grows linearly with r.

By this method of correction it was found that irregularities are considerably smoothed down. The only exceptions are rural villages near towns, where the effect of the town is smaller than that calculated from its number of inhabitants, presumably because there is no great admixture between rural and urban poplations.

A graphic representation (Fig. 3) shows a rather good fit of $p(r)$ with the formula $e^{-k\sqrt{r}}$ for all points except, unfortunately, the most important one, namely, the marriages of the parish villagers between themselves. As this is a major disadvantage unless it can be further corrected, there is nothing to recommend this representation. It was, however, useful here in pointing out that when the individual parishes are analyzed individually, the rather remarkable result is observed that all the parishes show practically the same slope of the curve. Thus, although some of the parishes are from mountainous regions, others from perfectly flat country,

[2]"Distance" here means the average distance from the center of the points in the area comprised between two circles, whose radii are the class limits of the distance considered. Road distances are used throughout.

mobility as expressed per kilometer person is almost the same for every parish. This is indirectly shown in Figure 3 by the ranges of individual parish values, which are relatively small and almost constant throughout. It will be noticed that the range of the first point (for $r = 0$) falls off the line; the range of the second point

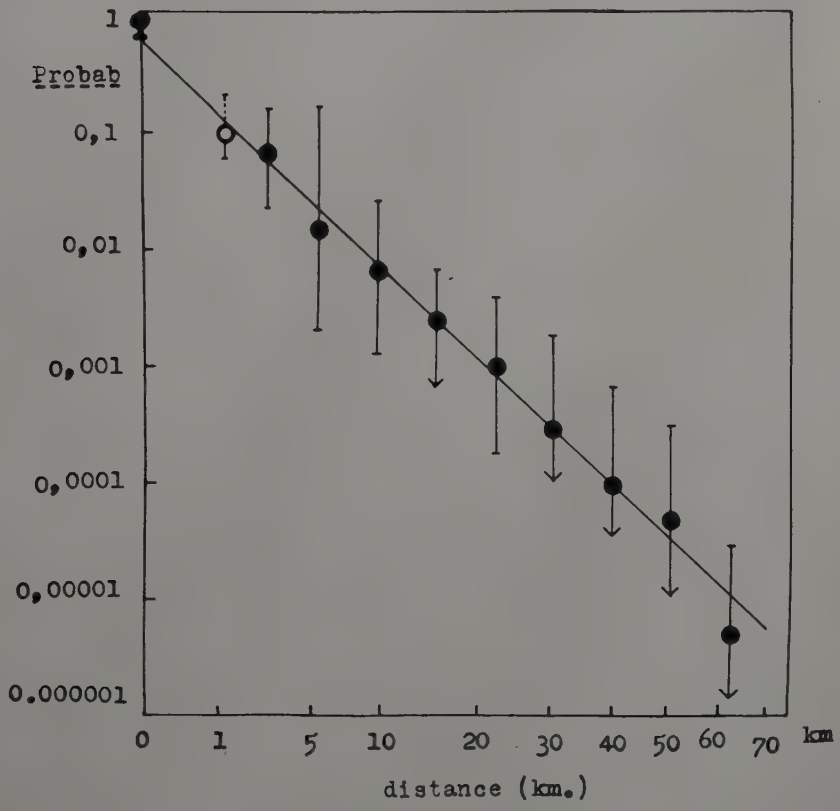


FIGURE 3. Matrimonial migration. Fitting : $e^{-k\sqrt{\text{distance}}}$

is smaller because only five parishes out of the twenty-one here examined have neighboring parishes situated at such a small distance (1–2 km.). For this reason this datum is given as a white circle in Figures 4 and 5. In fact, the error of this point is larger than the error of the others although the (rather fallible) measure of variation employed in the graph, the range, is smaller.

A more interesting representation stems from a straight application of the formula used to describe gravitation, or the attraction between electric charges, as well as the effect of distance on some social phenomena. According to this formula the "attraction force" of an individual of a village situated at distance r, and hence the probability of marriage is directly proportional to the number of people living in the village, and inversely to the square of the distance.

On the Parma data the fit of such a hypothesis is good for the shorter distances (Fig. 4), but poor for longer ones (which, however, contribute less than 5 per cent of the total marriages).

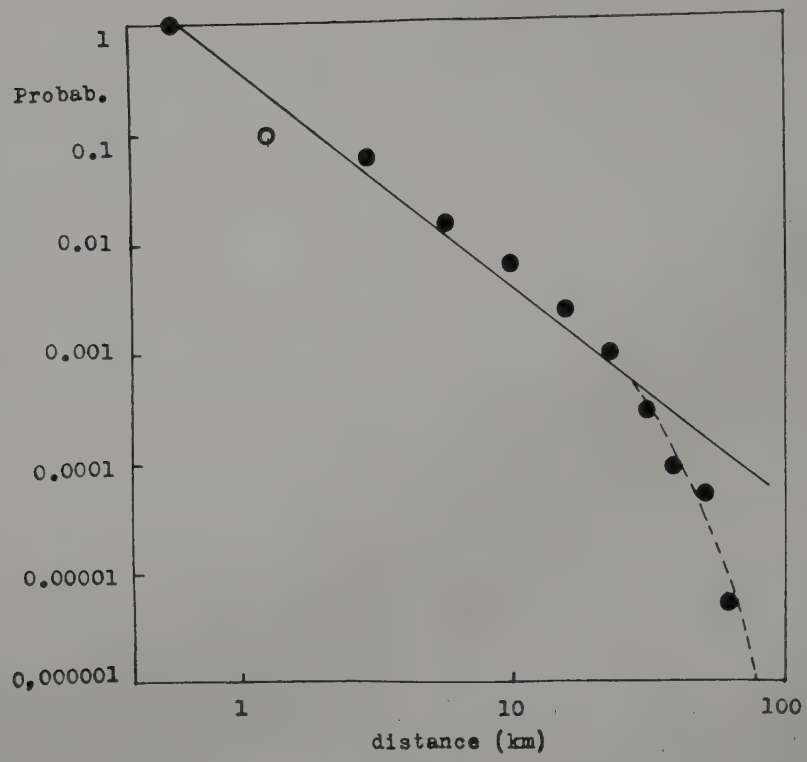


FIGURE 4. Matrimonial migration: the gravitational model applied to the data of Figure 2.

The data on the Swedish parish of Ovanåker (collected by Fraccaro and communicated at this Congress), which I have been able to examine, fit the curve in a remarkable way, and so do short-range data collected in the city of Philadelphia (Bossard, 1932).

In formulae, the attraction of an individual and, therefore, the frequency of marriage at distance r will be

$$f = \frac{k' A}{r^2};$$

$$\text{therefore } \frac{f}{A} = p = \frac{k'}{r^2}$$

where A is the number of people living at distance r. For a population homogeneously distributed over an area, $A = 2\pi rd$ where d is population density and therefore f is proportional to d/r.

One practical difficulty which is encountered in fitting a law of "Newtonian" attraction is that of determining the distance of an individual from his own village, in order to estimate endogamy. I have tentatively used half the radius of the parish taken as a circle, as is sometimes done for the calculation of population potentials in demography (Stewart, 1947). This last concept stems in fact from essentially the same considerations as those used in testing the Newtonian type of attraction.

Actually, the Newtonian model is interesting because it is somewhat opposite to that of diffusion. The gravitational model is based, in fact, on a dynamic equilibrium between villages. With diffusion, on the other hand, the effect of distance terminates at equilibrium, at which any person can mate with any other person with constant probability irrespective of distance. If a bachelor allows enough time to elapse before marrying, or if mobility is very high, diffusion can proceed a long way before marriage takes place, and eventually complete panmixia is reached at the limit of infinite time or mobility.

Actually, some hints are available that diffusion does play some role. If we examine the correlation between age at marriage and distance between the place of residence of the spouses, we find an effect which suggests that the older the spouses, the greater the distances between their birthplaces (or residences) since they have had more time to move around. Here the analysis has been carried out in a preliminary way, only to the extent of camparing the average age of brides or bridegrooms in endogamous and hexogamous marriages (Table I). The effect is significant but small. It is possible that a better model should be a compromise between a gravitation theory and a diffusion theory.

TABLE I

MEAN AGE AT MARRIAGE
(20 villages, 1800-1950)

	Wife %	Husband %	No. of marriages
Both from the village	24.72	28.71	1,077
Husband from elsewhere	25.09	30.11	972
Wife from elsewhere	27.19	30.26	31
Both from elsewhere	26.05	30.37	95
Resident in the parish	24.89	28.75	
Resident elsewhere	26.33	30.13	
Difference	1.44±0.55	1.38±0.30	

However, coming back to our primary interest, that of estimating migration, we do not have a perfectly satisfactory formula, but a single parameter formula similar to $e^{-k\sqrt{r}}$ or kA/r^2 can probably represent the situation, or at least extract most of the interesting information. If a single parameter formula is eventually found to be valid for representing the data, any particular estimate of migration will be a simple function of this parameter and, if properly estimated, any particular parameter chosen to indicate migration will be a function of all the others. Of the estimates presently available, at least for the purpose of making internal comparisons in our set of data, "endogamy" E, or the frequency of marriages in which both partners belong to the same parish, is convenient. Besides extracting a fair amount of the information, it also has the advantage that m is easily calculated from it for the island model, taking the parish as the unit cluster ($m = 1 - \frac{1}{2} E$). This will be an underestimate of m if the contribution to migration of the changes of residence, other than those connected with marriage alone, are frequent, a fact which demands a closer scrutiny in every case. From a preliminary exploration it is not important in our case.

When E values (x) for twenty-one parishes of the Parma valley are correlated with three ecological variables: village size (y), local population density (w), and

altitude (z), some interesting results are obtained (Table II). E is directly correlated with village size when the effects of density and altitude are eliminated ($r_{xy.wz} = +.66$). There is a negative correlation with density which persists after elimination of village size ($r_{xw.y}$) though it is reduced to a non-significant amount after elimination of density. However, density and altitude are here highly correlated, in an inverse way, in this sample ($r_{wz} = \sqrt{.84}$) and in the area in general, so that almost all that is stated of one is inevitably found to be true of the other variable as well. One exception is, however, the correlation of E with altitude which persists after elimination of density effects ($r_{xz.y} = +.82$ and $r_{xz.yw} = +.57$).

TABLE II

ENDOGAMY (PERCENTAGE OF MARRIAGES IN WHICH BOTH PARTNERS RESIDE IN THE SAME PARISH) IN 21 PARISHES AND ITS CORRELATION WITH DEMOGRAPHIC AND ECOLOGICAL DATA

	Variables	Mean	Range
x	Endogamy	54.5%	34–77%
y	Village size	700	159–2,495
w	Pop. density	86.5	37–180
z	Altitude (m.)	478	60–950

Correlation coefficients

Zero order	First order	Second order
$r_{xy} = +.25$	$r_{xy,w} = +.52^*$	$r_{xy,wz} = +.66^{**}$
	$r_{xy,z} = .67^{**}$	
$r_{xw} = -.67^{**}$	$r_{xw,y} = -.76^{**}$	$r_{xw,yz} = -.34$
	$r_{xw,z} = -.28$	
$r_{xz} = +.66^{**}$	$r_{xz,y} = +.82^{**}$	$r_{xz,wy} = +.57^*$
	$r_{xz,w} = +.25$	
$r_{yw} = +.20$		
$r_{yz} = -.34$		
$r_{wz} = -.84^{**}$		

*Significant at 5% level.
**Significant at 1% level.

While the conclusions thus reached deserve to be further tested and amplified by an increase in the size of the sample of parishes, it is interesting that the conclusion on correlation of E with village size is in agreement with the expectations from the gravitational model. The model taken in its simplest form does not directly give information on the possible effects of altitude and density, unless these environmental parameters are further analyzed, as is done below.

The effect of altitude is worth observing on the whole distribution of distances. Subdividing the twenty-one parishes into two groups, those located above 400 meters and those below, and taking the frequency of marriages $f(r)$ per kilometer in the two groups (Table III), one finds that the mountain parishes have a lower mobility at short and intermediate ranges though a slightly higher one at far distances. Lower mobility of the mountain population is a fairly obvious result as one mile on a mountain path takes much more time and effort to travel than a mile on a regular road in a hilly or flat country. However, the difference in mobility is not as striking as one might have expected. Moreover, as already noted when analyzing Figure 3, there is no important variation between parishes when mobility is given per kilometer person rather than per kilometer. This point can be confirmed in particular for the comparison between averages for mountain and plains parishes.

Another factor likely to play a role is the fact that in the mountains the popula-

tion is more often concentrated in villages. The fraction of population living in "isolated houses" as opposed to clustering of houses and minor or major centers increases strongly with population density (and, therefore, decreases strongly with altitude) (Table IV).

TABLE III

MATRIMONIAL MIGRATION IN A GROUP OF 10 PARISHES AT LOW ALTITUDE (P) AND A GROUP OF 11 PARISHES AT HIGH ALTITUDE (M).

Distance (1 unit = .625 km.)	P (%)	M (%)
0–2.5	51.3	64.2
2.5–6.5	9.9	9.9
6.5–12.5	15.2	6.6
12.5–20.5	9.9	6.3
20.5–30.5	6.0	3.9
30.5–42.5	3.7	1.8
42.5–56.5	1.0	1.8
56.5–72.5	0.7	1.3
>72.5	2.0	3.6
TOTAL NUMBER OF MARRIAGES	5,604	3,060

$\chi^2[8] = 287.6$.

TABLE IV

DISTRIBUTION OF POPULATION IN CLUSTERS AS AFFECTED BY DENSITY

Density class	Average density	No. of villages ("frazioni")	Median size of villages	Village size/density	Percentage of population living in isolated houses
0–50	44	70	300	6.8	11.1%
51–95	66	62	475	7.2	25.5%
96–140	122	74	680	5.6	47.4%
141–180	157	56	915	5.8	65.8%

It was also tested whether the spacing between villages is increased in the mountains; a possibility which would be important if the gravitational model is correct and could help explain the higher rate of endogamy observed there. In fact, in this case, if two villages, each of 1,000 inhabitants, were at a distance of 10 km. they would, with the law of the square of distance, be more endogamous than four villages each of 500 distributed regularly over a total distance of 10 km., in such a fashion as to give the same population density. However, it is found that the spacing of villages is almost independent of population density; the lower population density being almost exactly compensated by the smaller average size of the village and, therefore, their higher number per unit of area (Table IV). Moreover, a fraction of the mountainous area is totally uninhabited, and villages may even turn out to be a little closer one to the other in the mountains than in the plains. An exact comparison has to take account, however, of the different spatial distribution in the two cases (see Table VII), a fact which by itself has a relevance in the present connection.

Consanguinity Data

The classical approach to the study of population structure in man has been through consanguinity studies (for a recent review, see Freire-Maia, 1957b). The files of dispensations existing in the archives of Catholic bishoprics have formed a favorite material for this kind of investigation.

One caution that is perhaps not widely appreciated is that dispensations thus collected form a list of planned marriages, not of marriages actually performed. The extent of the discrepancy is now being evaluated and may be relatively large, perhaps in view of the delay, especially in earlier generations, between requesting and obtaining a dispensation.

The main difficulty attached to drawing conclusions from consanguinity data as regards population structure is that of non-randomness in the sense of a more or less strong bar against consanguineous marriages, which is posed by tradition and civil or religious customs. An attempt is being made to evaluate the effect on the closer consanguineous marriages.

Although objections can be raised against it, the study of consanguinity in man offers too tempting an approach to be neglected, in view of the availability of data.

Figure 5 shows a synthesis of data from consanguinity dispensations from 1851 to 1950 existing in the archives of the Parma Diocese. The 295 parishes belonging to the diocese have been divided into sixteen categories, according to four classes of population density (of the comuni to which each parish belongs), and four classes of village sizes. The bars in Figure 5 represent the average value of F (Haldane and Moshinski, 1939). The fractions of which each bar is composed show the contributions to F of the various types of consanguineous marriages, or more precisely the marriages with given coefficient of inbreeding, as shown by the illustrative bar on the right of the figure.

It is clear that population density has a high inverse effect on F, as has been shown before for Brazil by Freire-Maia (1957a). Village size has also had some effect, though smaller than in the partial collection of these data previously published (Cavalli-Sforza, 1956), and relating to the last quarter of the century, the examination of which has not been completed.

It is also clear that the contribution of marriages, other than those of first cousins, is different in the various classes of population density, being about a half of the total in the zones of low population density and one-fifth in those of high density, with lower F value.

One use which can be made of consanguinity data in order to evaluate properties of populations is to calculate the so-called "isolate size" of Dahlberg (N_D). This is the number of people among whom a spouse is selected and is based on the observed frequency (c) of marriage between relatives of a given kind. Assuming that the choice between a consanguineous and a non-consanguineous mate is a random one, and knowing how many relatives of a given kind each individual has, or is expected to have on the average (b), the number of people among whom a spouse is selected (N_D) can be obtained from $c = b/N_D$. The original formulae by Dahlberg have been recently improved by Frota Pessoa (1957) so as to take into account the effect of the variability in number of fertile individuals per family. However, as these formulae require knowledge which is not easily available and the approximation obtainable is, for reasons to be discussed later, influenced proportionately more by other uncontrolled factors, I have used for calculation the standard formulae.

The estimates of "isolate sizes" obtained with the Dahlberg method from these

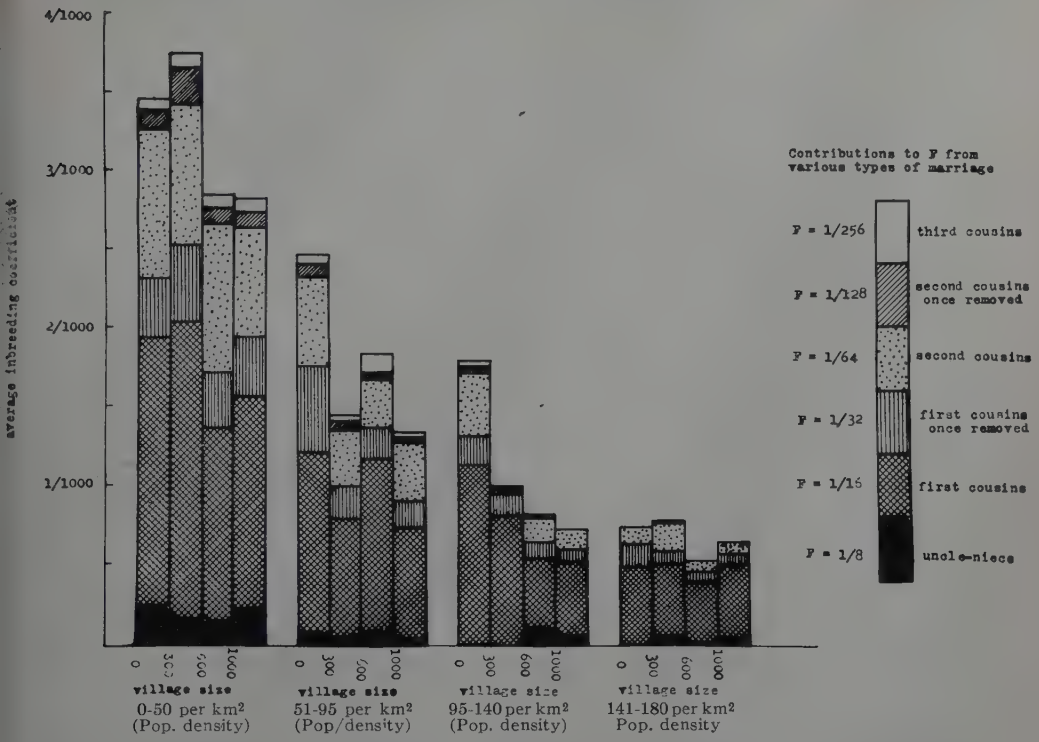


FIGURE 5. Average inbreeding coefficient as a function of population density and village size.

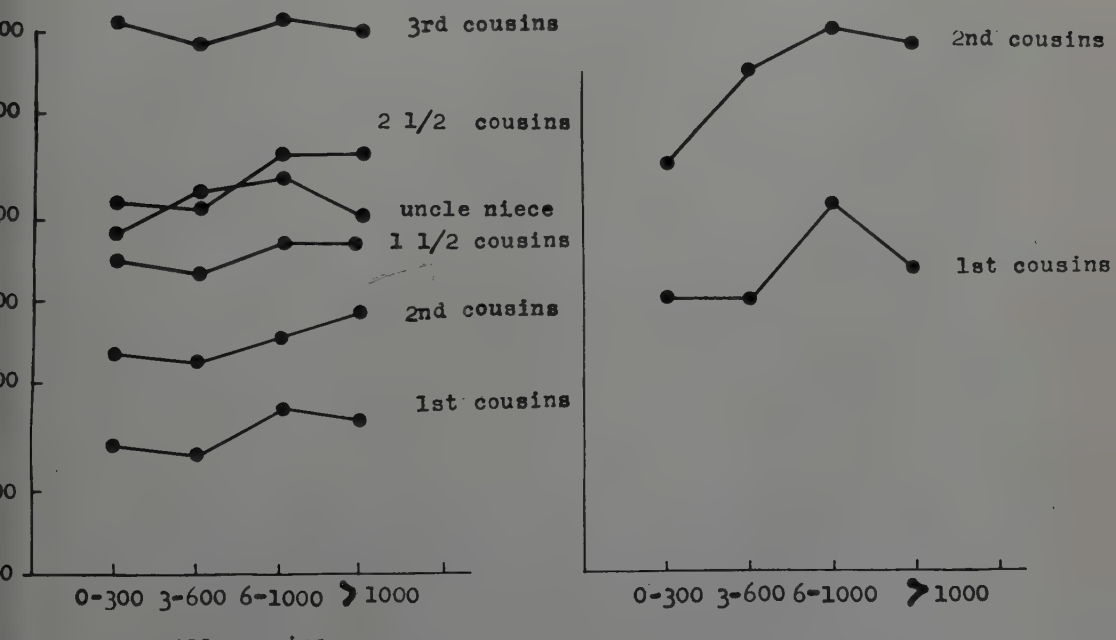


FIGURE 6. *Left*: Low population density; *right*: high population density.

data are given graphically in Figure 6 in such a way as to show how they vary with village size, type of marriage, and population density. Only the two extreme density classes are given, but the intermediate ones yield intermediate results. The values are actually calculated not from the frequencies of the indicated marriage types, but from the frequencies of consanguineous marriages with the corresponding degree of inbreeding; the difference, however, is trivial. Some discrepancy is noticeable between these calculations and the earlier ones (Cavalli-Sforza, 1956) which were based on the last quarter of the century here considered. These discrepancies are to be further analyzed in future work.

In general it can be concluded that: (1) the estimates of isolate sizes increase with population density; (2) the effect of village size, if any, is modest (the mode of graphic representation is such that if there were proportionality between isolate size and village size the curves should be straight lines at a 45° angle); (3) of the various types of consanguineous marriages, first cousins give the lowest estimates, and cousins of other degrees progressively lower estimates, probably because they are progressively more influenced by migration. In fact, the more generations there are between the common ancestors and the consanguineous individuals the greater the amount of migration, and therefore of diminution of the number of consanguineous people in the immediate neighborhood; (4) degrees of relationship involving an unequal number of generations, such as uncle-niece (or aunt-nephew) or cousins once removed, give higher estimates of isolate size because of their comparatively lower frequency. This is probably to a large extent the result of the unfavorable age relation between such relatives, and naturally this effect tends to cancel out with increasing remoteness of the relationship.

The concept of "isolate size," or at least its estimates, are therefore to be taken with caution if they are to be considered at all. But unquestionably these estimates can become more useful than they are now if it is possible to remove disturbing effects, such as those of age and of migration. A step in this direction can be provided by a more detailed examination of the data at the level of distinguishing classes of consanguineous marriages of the same general type, according to the sex of the ancestors intermediate between the common ones and the consanguineous mates. There are, for instance, four classes of first cousins and sixteen of one and a half or second cousins, and they have been shown in other situations to have discrepant frequencies (for example, Morton, 1955). This is true also of the present data, but the relative frequency of the various types differs with time and space, and the problem will require a special analysis.

More difficult to remove is the effect of non-randomness, which derives from traditional bars against consanguineous marriages whenever they are incomplete and ill-defined, but an attempt could perhaps be made at estimating their strength.

When the factors mentioned have been duly analyzed and their effects assessed it is likely that a clearer picture may emerge from consanguineous data.

Gene Frequencies

The ultimate goal of population genetics is naturally that of being able to make predictions about frequencies of genes, genotypes and phenotypes, their distributions, trends and equilibria. Clearly the direct estimation of gene frequencies whenever possible gives more direct and more satisfactory information than any other method, but may, if taken without consideration of the demographic data, be insufficient and even misleading.

A sampling survey of blood-group gene frequencies has been undertaken in the area which has formed the object of this investigation, and exactly in that section of it which is defined by the valley of the Parma River, which occupies the eastern third of the area. Approximately an equal number of adults was sampled from each parish. Out of the ninety parishes only fifty-three have been sampled so far and the data subjected to a preliminary statistical analysis. While waiting for the more complete data, certain tentative conclusions are worth mentioning.

Two types of analysis can be used on the data that were collected. One is the examination of whether genotype frequencies are in agreement with the equilibrium values expected under random mating, or the particular mating conditions of the population. The other is the analysis of the variation in gene frequencies over the geographical area which is being examined.

Analysis according to the first approach has not been completed. However, the distribution of phenotype frequencies for the blood-group systems ABO (A_1A_2B) and MN was tested for the goodness of fit of the Hardy-Weinberg equilibria in individual parishes. When the results are pooled for all parishes, some significant departures from expectation are found, especially for the MN system, in that a slight excess of heterozygotes exists.

A fairly clear-cut result has been obtained by the second approach. For this analysis the available data were grouped in a hierarchy as follows. Adjacent parishes showing closer geographical relations were assigned to groups (called the first order), containing usually two or three parishes. These groups were further classified into seven major groups (of second order), which correspond to the four southern mountain "comuni," Corniglio, Monchio, Tizzano, Palanzano, the intermediate "comune" of Calestano,[3] and two areas forming respectively the hilly and flat parts of the country which descends north of the Apennines towards Parma town.

A hierarchical analysis of the variation between parishes for the frequencies of genes A, B, M, R_1, R_2, and r yields the results summarized in Table V. A fair amount of variation is encountered, and almost all the heterogeneity chi squares (between parishes within groups of first order) are significant, and so are a number of those for the higher categories.

However, the heterogeneity between groups of first order and between groups of second order cannot be considered proved by the significance of their relative chi squares, because of the existence of heterogeneity at the lowest level. Rather, chi-square analysis for the higher levels of hierarchy must, as a consequence of such heterogeneity, be transformed into an analysis of variance. When this is done (Table V, F values), none of the heterogeneity at the higher level remains significant. As a first approximation we can therefore conclude that in this area there is variation above that expected from random sampling, but it is mostly confined to differences between adjacent parishes and no clear gradients or pockets of gene frequencies are observed. Variation is, in a word, confined to a microgeographical level.

When the total variation is subdivided according to the groups of second order, it is found that significant heterogeneity is entirely confined to the four parishes in the mountains, with no special preference for any blood groups (except perhaps blood group A for which the variation is smaller), while the flatter part of the region seems perfectly homogeneous in regard to blood groups.

[3]Discarded in Table VI because of the paucity of data.

TABLE V

BLOOD-GROUP GENE DISTRIBUTION: VARIATION IN GENE FREQUENCIES

System	Gene	Variation between adjacent parishes within groups of first order	Variation between groups of first order within groups of second order		Variation between groups of second order	
ABO	*A*	$\chi^2[28] = 34.62$	$\chi^2[18] = 31.22$*‡	F = 1.40	$\chi^2[6] = 10.20$††	F = 1.20
	B	51.68**	37.85**	1.14	11.31	1/1.03
MN	*M*	66.43**	47.10**	1.10	15.21*	1.04
	R_1	39.38	19.38	1/1.30	9.36	1.22
Rh	R_2	48.26**	22.01	1/1.38	15.23*	1.65
	r	43.60*	14.94	1/1.89	4.18	1/1.60

*P <5% **P <1% ‡df 18,28 ††df 6,46

Since the mountainous part of the area is the one in which lower density, smaller villages, less migration, etc. prevail, this result is far from surprising, but the magnitude of the variation encountered is somewhat astonishing. Although some of the variation, for example, that of the Rh genes, is probably partly due to the use of inefficient methods of estimation of gene frequency, to be improved in the later and more complete analyses, no cause has been found which might have artificially increased this variation. While these data can, therefore, be taken to constitute *prima facie* evidence of drift, no possibility exists so far of discarding local differences of selection, although the variation would require a microheterogeneity of selective conditions which may be difficult to imagine. There is a chance of testing this further by analyzing the heterogeneity of the Hardy-Weinberg equilibria among parishes.

However, instead of limiting the analysis to the picture given in Tables V and VI, one may try to carry it further and estimate effective sizes from the observed variation in gene frequencies, using the formulae given by Wright.

TABLE VI

BLOOD-GROUP DISTRIBUTION χ^2 BETWEEN PARISHES FOR GROUPS OF SECOND ORDER

	Mountain area				Low area		Sums	
	Corniglio (df 7)	Monchio (df 9)	Tizzano (df 5)	Palanzano (df 3)	Hills (df 12)	Plains (df 10)	Mountain (df 24)	Low area (df 22)
A	16.01*	10.32	21.04**	5.39	17.76	5.64	52.76**	23.40
B	4.79	41.31**	11.88*	4.40	10.04	18.11	62.38**	28.15
M	17.17*	46.58**	13.01*	3.37	19.45	13.95	80.13**	33.40
R_1	10.46	14.77	6.49	2.69	8.88	15.47	34.41**	24.35
R_2	17.67*	13.24	4.20	8.33*	12.93	13.10	43.44**	26.03

*5% significance level.
**1% significance level.

As only the mountains show heterogeneity between parishes, the analysis will be limited to them, subdividing the parishes into two groups, smaller and larger in size, to test the effect of village dimensions. The island model will be used, con-

sidering that: (1) in the mountain area the degree of clustering is very high; (2) the spatial distribution of people is somewhat intermediate between the two types offered by the continuous model, namely the linear, and the area distribution (Table VII), so that the choice between them would be difficult; and (3) there being no clear-cut macrogeographical variation in gene frequencies over the area, immigration from neighboring groups gives on an average almost equivalent results as immigration from more remote groups. This corresponds to the expectation of the island model.

TABLE VII

PATTERN OF GEOGRAPHICAL DISTRIBUTION OF PARISHES†

	Low altitude (<400 m.)			High altitude (>400 m.)		
		Expected			Expected	
Distance	No. of parishes	Area distribution	Linear distribution	No. of parishes	Area distribution	Linear distribution
1.5–2.5	1	3.1	17.0	4	2.8	15.3
2.5–6.5	32	27.8	68.0	47	25.0	61.3
6.5–12.5	101	88.1	102.0	73	79.3	91.9
12.5–20.5	189	204.0	136.0	167	183.8	122.5
	323	$\chi^2_{[3]} = 5.0$	$\chi^2_{[3]} = 54.8^{**}$	291	$\chi^2_{[3]} = 21.9^{**}$	$\chi^2_{[3]} = 31.7^{**}$

Difference between distributions at low and high altitudes
$\chi^2_{[3]} = 8.82^{*}$

*P < 5%
**P < 1%

The spatial distribution of villages is analyzed by counting, for each of the twenty-one parishes, how many parishes are found within circles at the distances given in the table. Here, as elsewhere, distances are road distances. The frequencies of parishes thus counted are cumulated for the various parishes distinguished into two groups, namely mountain and plains. The observed frequencies are then compared with those expected for a linear and an area distribution.

From the smaller parishes the variation in gene frequency σ^2_P, after elimination of the sampling component, gives rise to estimates of N which, for $m = .15$, range between 75 and 230 (for various blood-group genes). The average village size is 233 and from it a "demographic" effective size can be estimated as 205. This demographic estimate is higher than the genetic one and any estimate from consanguinity data would be even higher. It should be noted that any demographic estimate so far obtained is vitiated in the excess direction by failing to consider social differences within the population. All consanguinity estimates are vitiated usually in the same direction, and especially by non-randomness of marriages. The estimate of N from the variation in gene frequency may seem low, especially if one thinks that the existence of selection for blood-group markers must tend to raise it above the "true" unknown value.

Data from the larger mountain parishes give similar, and occasionally even lower estimates, but the magnitude of the error involved in estimation makes comparisons difficult at the present stage of the investigation.

However, it has been noted from Dahlberg's "isolate sizes" that these estimates do not increase proportionately with village size. Possibly the increase of village size is accompanied by an increase of social complexity which keeps "effective sizes" low. Also, m decreases somewhat with village size. The data of Table VII

suggest, however, that the pattern of geographical distribution is likely to be an important factor of gene variation in this area. An assessment of its relative importance is left for future investigators.

In conclusion, the study of population structure in man is probably not as direct a task as it may seem, in spite of the availability of many demographic data. I am still convinced, however, that man is the organism of choice for many such problems. It would be especially desirable—an opinion which I believe I share with some other people—if these studies were extended to a variety of primitive populations, while they are still primitive, although clearly, the more primitive the subjects of study are, the less amenable they will be to demographic investigations.

Summary

1. On the premise that man is a "good" organism for the study of population structure, an investigation has been started in an area chosen for the variety of physical environment it offers and the availability of good demographic records, the Parma Diocese in Italy. An analysis is being carried out of (*a*) clustering and geographical distribution of the population; (*b*) intermarriage, and genetic migration; (*c*) consanguinity; (*d*) distribution of blood group genes.

2. Of the various orders of clusters of people that can be considered as "villages," the "parish" (about 575 inhabitants on the average) is the most satisfactory one for a first approach. "Effective size" in Wright's sense is about 88 per cent of the village size, but this "demographic" estimate constitutes a very rough approximation.

3. Genetic migration is here estimated from the distance between the residence of the two spouses. This is only a fraction, but an important one, of the total migration which it is desired to estimate, that is, the distance between birthplaces of parent and offspring.

4. The distribution of matrimonial distances does not fit the normal diffusion model, but, as already shown for a number of other organisms including man, it shows a better fit with the empirical formula $e^{-k\sqrt{r}}$ (r = distance).
of villages of N individuals at distance r is proportional to N/r^2.

6. The "attraction" does not vary for villages from different environments such as mountain or plains. In more general words, when the probability of marrying at distance r is weighed for population density at that distance, it is constant in the whole area investigated.

7. Diffusion when measured per kilometre is slightly higher, at short distances, in the plains than in the mountains.

8. "Endogamy" or the fraction of people who marry in the same village increases with village size and with altitude, and is relatively independent of population density by partial correlation analysis.

9. Consanguinity has a high dependence on population density and altitude, but it is very little, if at all, dependent on village size. Not only the quantitative, but the qualitative picture of consanguinity, that is, the relative contributions from marriages of various consanguinity levels, change with density.

10. As stressed by so many other authors, the Dahlberg formulae do not supply easily interpreted values of "isolate sizes." They show, however, that isolate size is almost independent of village size.

11. An analysis of the presently available data on blood group gene distribution shows that some groups of adjacent parishes have significantly different frequencies for blood group genes, irrespective of the blood group system investigated (ABO, MN, Rh). There is no major gradient or variation over the area: the variation is entirely "microgeographical" and confined to the mountain region (where isolation is highest).

12. The pattern of geographical distribution of parishes in the mountains is neither linear nor two-dimensional, but somewhat intermediate.

13. Estimates of "effective sizes" from variation in gene frequencies with the "island model" are somewhat smaller than demographic estimates even for the group of smallest villages, but, for a more satisfactory analysis, further data will be necessary.

14. In the bigger villages "effective size," in Wright's sense, in parallel with "isolate size" in Dahlberg's sense, is no higher than in the smaller villages. This probably reflects the fact that social stratification increases with village size, and tends to keep effective sizes low and almost independent of village size.

REFERENCES

BATEMAN, A. J. 1950. Is gene dispersion normal? Heredity *4*: 353–363.

BOSSARD, J. H. S. 1932–33. Residential propinquity as a factor in marriage selection. Am. J. Sociol. *38*: 219–224.

BÖÖK, J. A. 1956. Genetical investigations in a North-Swedish population. Ann. Human Genet., Lond. *20*: 239–250.

CAVALLI-SFORZA, L. L. 1956–7. Some notes on the breeding patterns of human populations. Acta genet., Basel *6*: 395–399.

DAHLBERG, G. 1929. Inbreeding in man. Genetics *14*: 421–454.

FRACCARO, M. 1958. Private communication.

FREIRE-MAIA, N. 1952. Frequencies of consanguineous marriages in Brazilian populations. Am. J. Human Genet. *4*: 194–203.

——— 1957a. Inbreeding in Brazil. Am. J. Human Genet. *9*: 284–298.

——— 1957b. Inbreeding levels in different countries. Eugen. Quart. *4*: 127–138.

FROTA-PESSOA, O. 1957. The estimation of the size of isolates based on census data. Am. J. Human Genet. *9*: 9–16.

GLASS, B.; M. S. SACKS; E. F. JAHN; and C. HESS. 1952 Genetic drift in a religious isolate: An analysis of the causes of variation in blood group and other gene frequencies in a small population. Am. Natur. *86*: 145–159.

HALDANE, J. B. S., and P. MOSHINSKY. 1939. Inbreeding in Mendelian populations with special reference to human cousin marriage. Ann. Eugen., Cambr. *9*: 321–340.

MORTON, N. E. 1955. Non-randomness in consanguineous marriage. Ann. Human Genet., *Lond.* 20: 116–124.

SKELLAM, J. G. 1951. Gene dispersion in heterogeneous populations. Heredity *5*: 433–435.

STEWART, J. Q. 1947. Empirical mathematical rules concerning the distribution and equilibrium of population. Geogr. Rev. *37*: 461–485.

SUTTER, J., and TRAN-NGOC-TOAN. 1957. The problem of the structure of isolates and of their evolution among human populations. Sympos. Quant. Biol. *22*: 379–383.

WRIGHT, S. 1943. Isolation by distance. Genetics *28*: 114–138.

——— 1946. Isolation by distance under diverse systems of mating. Genetics *31*: 39–59.

——— 1951. The genetical structure of populations. Ann. Eugen., Cambr. *15*: 323–354.

THE ROLE OF HETEROZYGOSITY IN *DROSOPHILA* POPULATIONS[1]

Bruce Wallace[2]

POPULATIONS of a species in which two or more phenotypically distinct forms occur sympatrically with relatively high and constant frequencies have been known for a long time. These are known as "polymorphic" species; the phenomenon is referred to as "balanced polymorphism" (Ford, 1940). Fisher (1930) outlined a genetic basis for the maintenance of a polymorphic system. The essential part of his argument lies in the selective superiority of heterozygous individuals; virtually any equilibrium frequency can be maintained in a population if the two homozygotes are to some extent at a selective disadvantage relative to the corresponding heterozygote.

The known cases of balanced polymorphism have increased in number since 1930. Not only have the recognized instances of phenotypic polymorphism been extended but numerous instances of "cryptic" polymorphism have been discovered as well. The latter cases include the numerous naturally occurring inversion systems in *Drosophila* and other Diptera (see Da Cunha, 1955), the cytologically altered chromosomes in grasshoppers (reviewed by White, 1954), and the chromosomal interchanges common in certain plant species (see, for example, Cleland, 1958).

For the purposes of the present discussion I will consider heterosis as equivalent to the selective advantage of heterozygous individuals which results in balanced polymorphism. Heterosis used in this sense corresponds to *euheterosis* as used by Dobzhansky (1952). I will not be concerned with the underlying mechanisms which give rise to heterosis; various possibilities are listed in Table I. It will be noted that heterosis *per se* can be explained as shown in the table on the basis of pleiotropic gene action which is equivalent in principle to the exploitation of diverse ecological niches or temporal fluctuations in environmental conditions; it can also be explained on the basis of properties of heterozygotes which are unique and which cannot be copied by either of the two homozygotes (Haldane, 1954; Robertson and Reeve, 1952).

At the present time there are probably more known—and better understood—instances of cryptic than of non-cryptic polymorphism. The so-called "cryptic" polymorphic traits, however, are really morphological traits made visible by cytological examination of chromosomes. The number of recognizably different, coexistent "cryptic" systems is frequently large. Dobzhansky and his co-workers in Brazil (Da Cunha, *et al.*, 1950; Dobzhansky and Da Cunha, 1954) have described a number of populations of *D. willistoni* in which the average number of inversion differences per individual is eight or more. The existence of these inversions appears to be correlated with the local diversity of ecological niches available to the species. Quite obviously the number of cytologically detectable polymorphic systems is limited by the chromosomal complexity which confronts the investigator, the clarity of the cytological picture which the species provides, and the limit of microscopic visibility. One may be excused, then, for speculating on the frequency of truly

[1]The work reported in this paper has been done under Contract No. AT-(30-1)-557, United States Atomic Energy Commission.

[2]Biological Laboratory, Cold Spring Harbor, N.Y., U.S.A.

TABLE I

SEVERAL POSSIBLE MECHANISMS BY WHICH HETEROZYGOUS INDIVIDUALS MAY ATTAIN HIGHER AVERAGE FITNESSES THAN THEIR CORRESPONDING HOMOZYGOTES

	AA	Aa	aa
1*	1	1	$1-s_1$
2	$1-s_2$	1	1
3	$1-s_3$	1	1
4	1	1	$1-s_4$
.	.	.	.
.	.	.	.
.	.	.	.
i	$1-s_i$ (or 1)	1	1 (or $1-s_i$)
Product	$1-s_A$	1	$1-s_a$

*The succession of numbers 1, 2, 3, . . . , i may represent: (*a*) different ecological niches within a single locality (Levene, 1953); (*b*) different environmental conditions prevailing in successive generations (Dempster, 1955); (*c*) different developmental processes governed by pleiotropic gene action (Caspari, 1950).

cryptic, heterotic systems; instances of heterosis at individual gene loci, for example, which are not detectable by presently available techniques.

In essence, one would like to estimate, if possible, the frequency of heterozygosity in terms of gene loci at which representative individuals of a population are heterozygous. It is entirely inadequate for this purpose to estimate the proportion of individuals heterozygous for genes at one or more loci; surely the proportion of such individuals approaches 100 per cent even in the absence of heterosis. Rather, what is needed is some technique for screening individuals with the purpose of determining the mean frequency of loci occupied by dissimilar alleles and the distribution of frequencies about this mean. At the same time, it would be desirable to exclude from the estimated mean frequency those instances of heterozygosis which arise passively from mutation pressure, from an influx of non-heterotic alleles by migration, and from relic alleles favorable at some earlier time but now in the process of being eliminated from the population.

Our experimental approach to this problem has consisted of an anlysis of the viability effects of radiation-induced mutations in heterozygous condition. Underlying our approach is the tacit admission that present techniques do not allow us to recognize all possible differences between alleles and, therefore, do not assure us of recognizing heterozygosity as such where it exists. On the other hand, we do have techniques which allow us to create individuals which are homozygous for genes at large numbers of loci (entire chromosomes or, if desirable, entire sets of chromosomes). We should be able, then, to draw inferences concerning the viability effects of these genes if we determine the average effect of newly induced mutations in heterozygous condition. We must assume, of course, that the new mutations are distributed at random among loci and represent an adequate sample of the array of alleles which might arise spontaneously at any locus.

The rationale for this type of approach is represented in Figure 1 by contrasting one's expectations under two extreme models—one based on the universal selective superiority of homozygous individuals and the other on that of heterozygotes. In

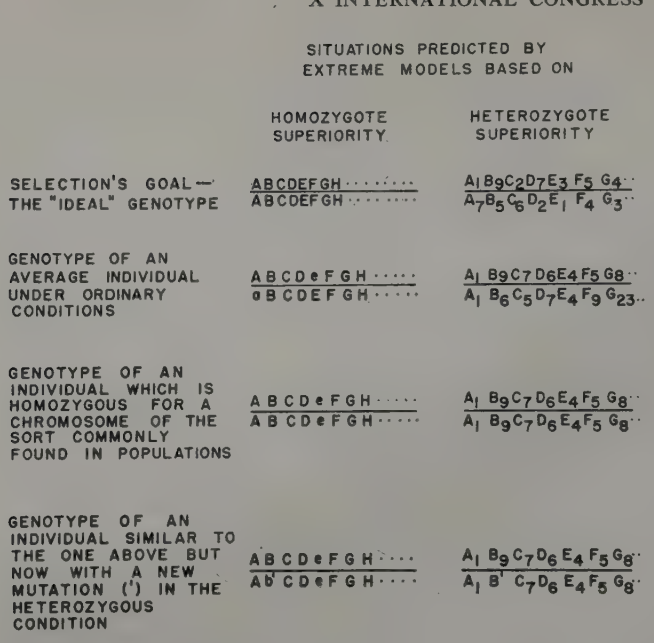


FIGURE 1. A schematic representation of expectations based on two extreme genetic models of population structure. A possibility for differentiating between these two models experimentally is outlined in the text.

the former we postulate that there is a "normal" or "type" gene at each locus, that mutant alleles are invariably deleterious when homozygous, and that heterozygous individuals never exceed the normal homozygotes in fitness or in viability. In the second model we postulate that at each locus there are a number of alleles which in heterozygous combinations give rise to "normal" individuals; the average fitness or viability of these heterozygotes exceeds that of individuals homozygous for any of the individual alleles. One need not postulate that every allele is capable of taking part in these heterotic combinations; certain mutations may be deleterious in virtually all gene combinations.

The first two entries under each theoretical model shown in the figure emphasize that the extreme situation predicted by either model (complete homozygosis of every individual for the "type" genes of a species or heterozygosity at every locus in the second model) does not exist in any real population. Homozygous superiority does not lead to complete homozygosity even in the absence of heterosis; mutations and alterations in selective pressures serve to maintain a small proportion of heterozygous loci. Similarly, in the absence of any mechanism for maintaining a series of unique alleles at every locus, some homozygosis must occur in a population even though heterozygotes are favored at each of these loci.

The third entry in the figure represents the genotype of an individual homozygous for a chromosome chosen at random from a population. It is well known for *Drosophila* that such individuals have an average viability lower than that of individuals heterozygous for two different chromosomes chosen at random. The lower viability of these homozygotes is predicted by both models. Even though selection may favor individuals homozygous for normal alleles, deleterious mutant genes are

sufficiently common that one or more of these is likely to be included among the genes carried by a given chromosome. The few that are included in this way are the limiting factors in determining the viability of individuals homozygous for an entire chromosome. The alternative model also predicts that individuals homozygous for an entire chromosome will be less viable than one heterozygous for two different chromosomes since, by definition, the genes present in the population have been selected on the basis of an average selective superiority of heterozygous individuals.

The last entry in the figure shows how one might possibly distinguish between the two models experimentally and decide which is more nearly correct. A homozygous individual is shown which is identical in all respects to that of the third entry except for the presence of a new, randomly induced mutation in one but not in the other of the two chromosomes. If selection favors "normal" homozygotes, most genes in a population will be represented by "normal" alleles; consequently, a new mutation induced at random will most likely be one that changes a normal gene to a deleterious allele. In order for a random mutation to improve the viability of the homozygous individual, the newly induced mutant allele must interact specifically and favorably with the few deleterious mutations already present or it must represent a back-mutation at a locus occupied by a deleterious allele. Both of these can be regarded as highly unlikely events. In contrast to these expectations, viability improvement as a result of a random mutation need not be a rare event if heterosis is a common phenomenon. First, no matter what locus on the chromosome is affected by radiation, the new mutation results in heterozygosis at that locus; this is a matter of definition. Second, the probability that this heterozygosity will increase the viability of the otherwise homozygous individual depends upon the relative proportions of alleles which act in a heterotic fashion or in a deleterious manner in combination with the original allele. Although the proportion of heterotic alleles may be small, one might hope, if selection does in reality favor heterozygous individuals, that it is large compared to the probability of getting a "beneficial" mutation under the alternative model.

An experimental technique for testing the effect of random mutations on viability is illustrated in Figure 2. A full description of this method has been published elsewhere (Wallace, 1958); for the present it is sufficient to give a simple outline of the system. In *Drosophila melanogaster* entire second chromosomes can be manipulated in breeding experiments by the use of a genetically marked (*Cy*, curly wings; *L*, lobe eye) chromosome carrying two large inversions which act as crossover suppressors. The *CyL* chromosome is maintained in a stock (*CyL/Pm*) whose other second chromosome is marked by the dominant gene *Pm* (plum eye color).

The upper portion of Figure 2 illustrates the isolation of a second chromosome from an experimental population (our population no. 18; see Wallace, 1956). Wild-type males from the population were mated with *CyL/Pm* females. Single *CyL*/+ F_1 males were backcrossed with *CyL/Pm* females. Several *Pm*/+ F_2 males and *CyL*/+ F_2 females of each culture were mated, brother with sister, to give F_3 cultures containing, among other types of flies, wild-type individuals homozygous for one of the second chromosomes carried by each of the original wild-type males. Four chromosomes which gave viable, fertile homozygous individuals were chosen from among those tested.

The center portion of Figure 2 illustrates how each of the four chromosomes was transferred by repeated backcrossing into the genetic background of our

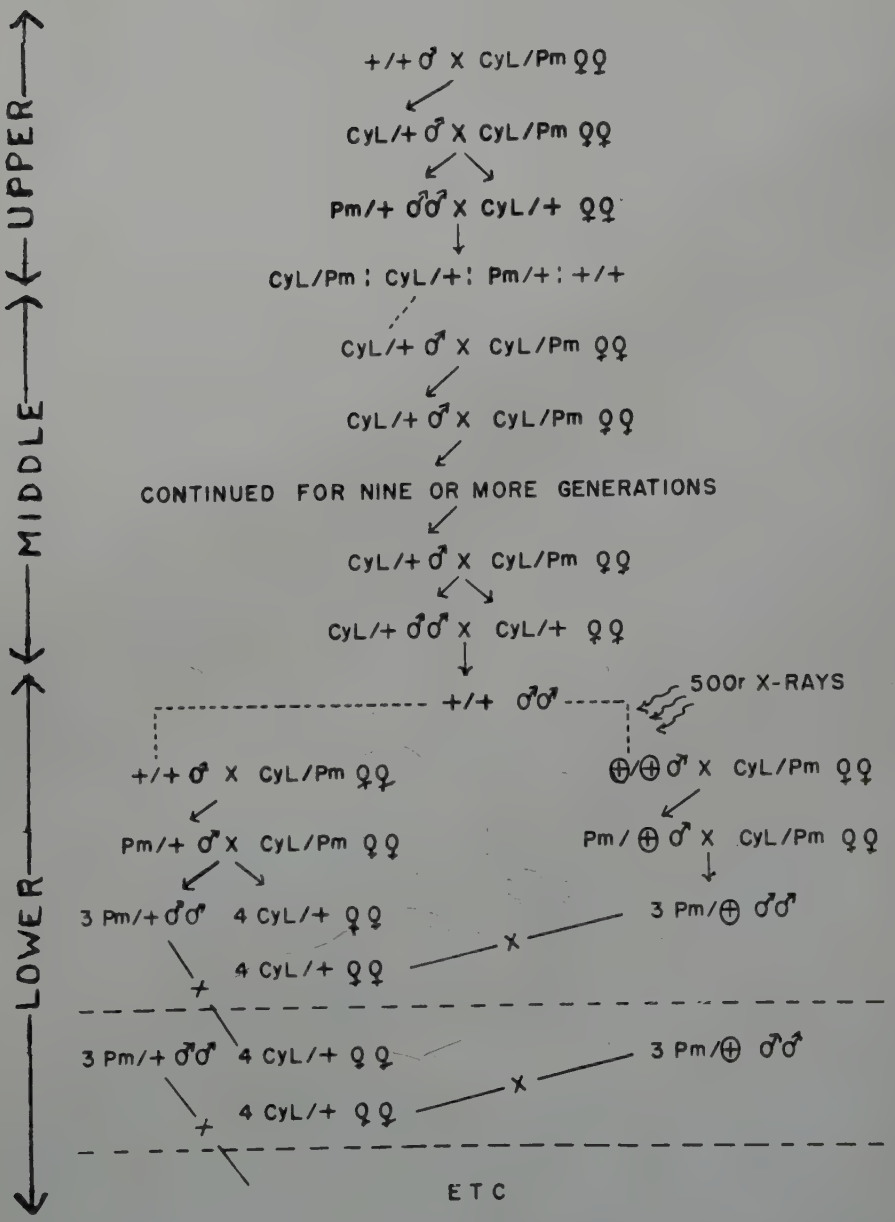


FIGURE 2. Mating scheme used to test the effects on viability of new mutations in heterozygous condition.

CyL/Pm stock of flies. Each generation a single *CyL*/+ male was chosen from each of the four chromosomal strains and these males were mated with *CyL/Pm* females. After nine or more generations of backcrosses it is most likely that all of the chromosomes other than the wild-type second were replaced by those of the balancer stock. The use of a single *CyL*/+ male in each of these crosses prevented an accumulation of a variety of wild-type second chromosomes in this material.

The lower portion of the figure illustrates the experimental crosses which lead to the evaluation of the viability effects of new mutations in heterozygous condition. Males homozygous for any one of the four chromosomes were obtained by mating *CyL*/+ males and females from one of the backcross cultures. These males (approximately four hundred in number) were divided into two groups of which one was exposed to about 500 r X-radiation. Both exposed and non-exposed males were mated individually with *CyL/Pm* females in vials. Single *Pm*/+ F_1 males from each vial were backcrossed to *CyL/Pm* females. In the irradiated material, three *Pm*/(+) F_2 males were obtained from each culture. (Irradiated wild-type chromosomes are designated as (+).) From the non-irradiated cultures, three *Pm*/+ F_2 males and eight *CyL*/+ F_2 females were collected. Four of the *CyL*/+ females of each culture were mated with *Pm*/(+) males of an X-ray culture; the offspring expected from these matings are *CyL/Pm*, *CyL*/(+), *Pm*/+ and +/(+) in equal proportions. The remaining four *CyL*/+ females of each culture were mated with *Pm*/+ males of a different control culture; the offspring expected from these matings are also *CyL/Pm*, *CyL*/+, *Pm*/+ and +/+ in equal proportions.

These two series of cultures allow us to determine the average effect of newly induced mutations in heterozygous condition. With the exception of those mutations induced by irradiation, the two series of cultures—control and X-ray—are virtually identical. Thus, differences in the amount by which the Mendelian ratios are distorted by differential viabilities of developing larvae in the two series can be ascribed to mutations induced by exposure to X-radiation.

The mating scheme outlined above can be—and has been—modified in different experiments so that (1) the *Pm*/+ flies of the X-ray series rather than the *CyL*/+ flies carried an irradiated chromosome, and (2) the chromosomes other than the second in the control cultures were exposed to radiation as were those of the X-ray series.

The results of seven large experiments involving the examination of more than nine thousand cultures and the counting of more than three and a quarter million flies are summarized in Table II. On the left are listed the average viabilities of CurlyLobe, Plum, and wild-type flies of the control and X-ray series in each of the different experiments; the viability of *CyL/Pm* flies is 1.000 in all cases. Although significant differences in the viability of +/+ and +/(+) flies were observed in only two of the individual experiments (2–9M, 15–22F), the viability of +/(+) individuals exceeds that of the control homozygotes in all six experiments. The over-all probability that the mean difference observed (1 1/2 per cent) is the result of chance is .002. (Note that the seventh experiment involves not homozygous wild-type flies but flies heterozygous for two different chromosomes.)

The relative viabilities of CurlyLobe flies in the X-ray series (*CyL*/(+)) are larger than those of their corresponding controls (*CyL*/+) in four of five experiments; in one experiment (2–9M) the difference is significant at the 5 per cent level. The over-all probability that the mean difference observed (0.9 per cent) is the result of chance is .04.

TABLE II

RESULTS OF SEVEN EXPERIMENTS IN WHICH THE VIABILITY OF INDIVIDUALS CARRYING RANDOMLY INDUCED MUTATIONS IS COMPARED WITH THAT OF THEIR NON-IRRADIATED CONTROLS

		A				B		
Experiment	C X	*CyL*/+ *CyL*/(+)	*Pm*/+ *Pm*/+	+/+ +/(+)	Number of cultures	*CyL*/+ *CyL*/(+)	*Pm*/+ *Pm*/+	+/+ +/(+)
2–9M	C	1.094	1.146	1.008	766	.955	1.000	.880
	X	1.115	1.137	1.033	764	.981	1.000	.909
6–14F	C	1.093	1.139	1.000	676	.960	1.000	.878
	X	1.108	1.140	1.007	672	.972	1.000	.883
15–22F	C	1.105	1.137	.989	636	.972	1.000	.870
	X	1.110	1.145	1.015	637	.969	1.000	.887
19–26M	C	1.100	1.143	.979	596	.962	1.000	.857
	X	1.108	1.136	.989	598	.975	1.000	.871
	C	CyL/+	Pm/+	+/+		CyL/+	Pm/+	+/+
	X	CyL/+	Pm/(+)	+/(+)		CyL/+	Pm/(+)	+/(+)
11–18M	C	1.098	1.127	.983	639	1.000	1.026	.895
	X	1.095	1.125	.990	637	1.000	1.027	.904
37–43M	C	1.154	1.189	.992	499	1.000	1.030	.860
	X	1.147	1.201	1.002	496	1.000	1.047	.874
	C	CyL/+	Pm/+	$+^1/+^2$		CyL/+	Pm/+	$+^1/+^2$
	X	CyL/(+)	Pm/+	$+^1/(+^2)$		CyL/(+)	Pm/+	$+^1/(+^2)$
23–34H	C	1.185	1.215	1.143	839	.975	1.000	.941
	X	1.182	1.222	1.155	837	.967	1.000	.945

A. *CyL/Pm*, not shown, has viability 1.000 by definition. B. Figures listed under "A" re-adjusted so that the class of flies (*Pm*/+ or *CyL*/+) of the X-ray series which is free of an irradiated second chromosome becomes the standard for comparing relative viabilities.

The viabilities of Plum flies heterozygous for non-irradiated wild-type chromosomes do not differ significantly between the two series in any single experiment or in the consolidated results of all experiments.

On the right side of the table we have adjusted the viabilities listed on the left so that the class of flies free of an irradiated chromosome in the X-ray series has been assigned in both the X-ray and control series a relative viability of 1.000. It can be seen once more that the +/(+) flies of the X-ray series have consistently higher viabilities than those of the controls. As for the *CyL*/(+) flies of the X-ray series, we now find that in two of the five experiments their relative viabilities are lower than those of the *CyL*/+ flies of the control series.

A full discussion of the additional tests to which these experimental data have been subjected will be given elsewhere (Wallace, 1958). For the moment we will simply mention that the two series of cultures, X-ray and control, did not differ significantly in mean culture size (mean difference of less than one fly in cultures whose average size was about 350 flies); classes of flies which were expected to have the same mean viabilities in the two series did in fact have the same viabilities; the mean variances of viabilities and even the distributions of these variances (Figure 3) of different classes and of numbers of flies per culture did not, with the

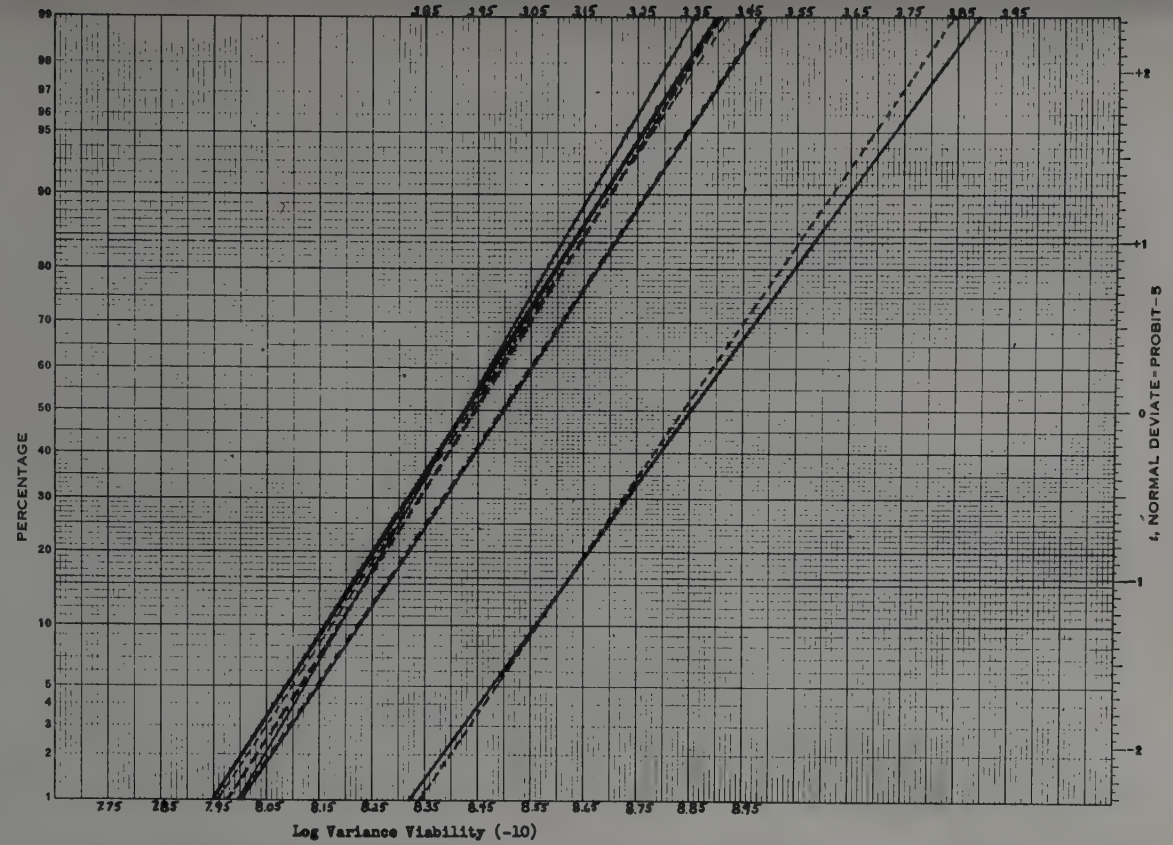


FIGURE 3. Distributions of log variances of relative viabilities of CurlyLobe, Plum, and wild-type flies (lower scale) and of numbers of flies per culture (upper scale). Variances are between cultures (usually twenty) counted by various individuals in each experiment. Dotted lines represent X-ray series of cultures; solid lines, control. The CurlyLobe and wild-type flies of both series of the two series have very similar mean variances; these are the four curves clustered at the left; heavy lines are wild type, light lines are CurlyLobe. The Plum flies of the two series are not expected to differ; these two curves are those which are nearly superimposed and which lie to the right of the four previous curves. The two curves for variances of numbers of flies per culture in the X-ray and control series are those at the extreme right.

exception of the $CyL/+$ and $CyL/(+)$ classes, differ significantly between the two series. The mean variance of the viabilities of the CurlyLobe flies did not differ but the distribution of variances for $CyL/+$ flies of the control cultures was somewhat more uniform than that of the corresponding class ($CyL/(+)$) of the X-ray series. We conclude, therefore, that the two series of cultures are comparable, that the larger proportion of wild-type flies in the X-ray series is real, and that this represents an actual increase in viability (or developmental rate) caused by the newly induced mutant alleles. We admit that these conclusions are tentative and that they may be modified by a more elaborate analysis of available data or by additional experiments. However, we can, on the basis of these conclusions, anticipate some of the more obvious alternative explanations of our results, explanations which avoid or restrict the role of heterosis.

There are a number of alternative explanations for these results which do not rely on heterosis. First, for example, one can claim that most radiation-induced mutations are dominant enhancers of viability. This possibility has been rejected because it raises problems more serious than the one it answers. It suggests that in large populations selection leads to the fixation of deleterious alleles at most loci but it gives no logical explanation why this should be so. Furthermore, it suggests that were we to test irradiated chromosomes in homozygous condition, we would find the same improvement in viability that we have seen in our experiments; this is contrary to the experience of radiation geneticists. We include in this rejected hypothesis the suggestion that the mutations we have induced improve viability of homozygotes because these are "abnormal" individuals. According to the model based on the superiority of homozygotes, an individual which is homozygous for a given chromosome differs only slightly from an individual heterozygous for two different chromosomes. To say that a random mutation improves the viability of homozygotes because these are "abnormal" is equivalent to claiming that specific, compensatory interactions are the rule and not the exception. An additional implication of this last argument is that the effect of mutations on viability is that of a regression towards the mean—individuals with poor viability would be improved and those with high viability would be harmed by new mutations; the average viability of individuals in a population would, in that case, represent a stable equilibrium based on mutation but virtually independent of mutation rates.

A second possible explanation for our data can be based on the recessivity of deleterious mutations and the dominance of mutations which improve viability. The average increase in viability observed in our experiments would, according to this scheme, result from the detection of dominant (not heterotic) viability modifiers only. This explanation, too, has been rejected. First, dominance and recessivity are not sufficiently complete to make this hypothesis entirely convincing. Second, even if dominance and recessivity were complete, the absolute minimum number of mutational changes per locus for any set of genes having a given effect on viability can be shown to equal $d\sqrt{(us)}$ where d is the observed increase in viability, u the spontaneous mutation rate, and s the reduction in viability characteristic of the genes of this particular set. By absolute minimum we mean that every induced mutation would be a mutation of the minus modifier present on the chromosome to its normal allele only. The number of mutations per chromosome demanded by this explanation appears to be unreasonably high even for material such as ours which has been obtained from a population whose past history includes chronic exposure to radiation.

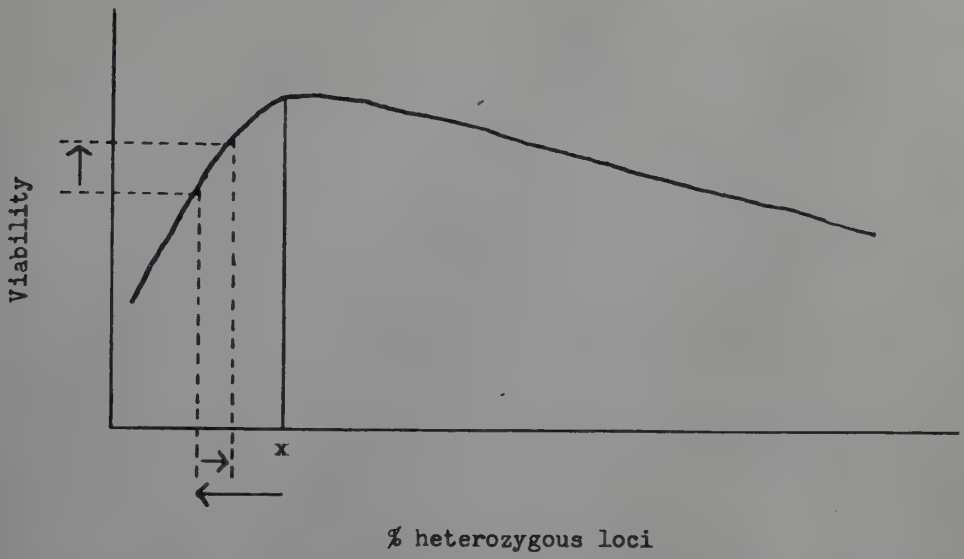


FIGURE 4. An illustration depicting how "optimal hybridity" might possibly explain the experimental results listed in Table II. If heterozygosis at x per cent of all loci is optimal, the viability of the homozygous control flies is lowered as indicated by the lower arrow. Induced mutations, by partially restoring the optimal proportion of heterozygosity (small arrow), might be expected to result in a slight increase in viability as well. See text for reasons for discarding this hypothesis.

A final alternative explanation for our results can be found in the postulate of "optimal hybridity" or "optimum heterozygosity," a postulate which admits the existence of heterosis but restricts it to a small fraction of all loci. According to this hypothesis our control homozygotes (+/+) had less than the optimal degree of heterozygosity and through irradiation we improved viability by partially restoring this optimum (Fig. 4).

Unless the above hypothesis is extended to include an optimum of 100 per cent heterozygosity, it too is rejected. X-ray induced mutations are presumably distributed at random among loci and, hence, there does not appear to be a fixed set of loci reserved for heterotic effects. If the optimal proportion of heterozygous loci is small, geographically isolated populations would most likely utilize multiple allelic series at different loci in maintaining this optimum within their individual members. The probability that exactly the same loci would be utilized by isolated populations would be negligible. One would predict, then, that inter-population F_1 hybrid individuals would have more than the optimum proportion of heterozygosity and as a result would be somewhat inviable. On the contrary, these inter-population hybrids are almost always more viable than are members of a single population; inviability, if it occurs in *Drosophila*-strain hybrids, is primarily a consequence of recombination in the F_2 and later generations (Vetukhiv, 1952, 1954, 1956; Wallace, 1955; Wallace and Vetukhiv, 1955).

A second version of the same explanation—optimal hybridity—is also rejected. In this version the optimal proportion of heterozygosity per individual is a low percentage which is met at the population level not by many alleles at a few loci

but by a low frequency of mutant alleles at many loci. This is rejected because of the improbability, should the number of heterotic alleles at each locus be restricted, that a random mutation will give rise precisely to that allele which produces the heterotic effect. It is rejected on the additional grounds that wild-type "isoalleles" are not uncommon in nature. Stern and Schaeffer (1943) found that of three wild-type alleles at the *ci* locus in *D. melanogaster* which they examined, each was demonstrably different from the other two. It is difficult to reconcile this finding with a predominantly common "normal" gene and a family of rare but heterotic mutant alleles at each locus.

As a result of the studies described above, we are adopting the tentative conclusion that heterosis plays a fundamental role in the genetic structure of Drosophila populations. We feel that at every locus there are heterozygous combinations of alleles which, on the average, give rise to individuals of higher viabilities or greater fitnesses than do homozygous combinations of the same alleles. Subject to the limitations imposed by chance elimination of alleles, by mating of close relatives, and by the finite number of alleles at a locus, we feel that the proportion of heterozygosis among gene loci of representative individuals of a population tends toward 100 per cent.

We suspect, though, that the heterotic effect exhibited by two alleles at one locus is not independent of the proportion of heterozygosity at other loci. It is difficult, for example, to imagine under the conditions of our experiments an increase in viability of 1½ per cent per locus (or per few loci) accompanying mutations at each of hundreds of additional loci. On the other hand, we do not visualize the change in sign for the viability effects of heterozygosity that the hypothesis of optimal hybridity seems to demand. That heterosis at a locus is a function of the background genotype has been suggested by the work of Robertson and Reeve (1955), Tantawy (1957), and Tantawy and Reeve (1956). These workers suggest that under a program of inbreeding it becomes increasingly difficult to attain homozygosis at unfixed loci as the proportion of fixed loci increases. Our experiments represent another approach to this question and appear to confirm the above suggestion for the improvement in viability accompanying the first increment of heterozygosity appears to be too large to accompany each additional increment.

REFERENCES

Cleland, R. E. 1958. The evolution of the North American Oenotheras of the "biennis" group. Planta *51*: 378–398.

Da Cunha, A. B. 1946. Polymorphism in natural populations of a species of *Drosophila*. J. Hered. *37*: 253–256.

——— 1955. Chromosomal polymorphism in the Diptera. Advance. Genet. *7*: 93–138.

Da Cunha, A. B., H. Burla, and Th. Dobzhansky. 1950. Adaptive chromosomal polymorphism in *Drosophila willistoni*. Evolution *4*: 212–235.

Da Cunha, A. B., and Th. Dobzhansky. 1954. A further study of chromosomal polymorphism in *Drosophila willistoni* in its relation to the environment. Evolution *8*: 119–134.

Dobzhansky, Th. 1952. Nature and origin of heterosis. *In* Heterosis: A Record of Researches Directed toward Explaining and Utilizing the Vigor of Hybrids, ed., J. W. Gowen, pp. 218–223. Ames, Iowa: Iowa State College Press.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Oxford: Clarendon Press.

Ford, E. B. 1940. Polymorphism and taxonomy. *In* The New Systematics, ed., J. S. Huxley, pp. 493–513. Oxford: Clarendon Press.

Haldane, J. B. S. 1954. The statics of evolution. *In* Evolution as a Process, eds., J. S. Huxley, A. C. Hardy, and E. B. Ford, pp. 109–121. London: Allen and Unwin.

ROBERTSON, F. W., and E. C. R. REEVE. 1952. Heterozygosity, environmental variation and heterosis. Nature *170*: 286.

——— 1955. Studies in quantitative inheritance. VIII. Further analysis of heterosis in crosses between inbred lines of *Drosophila melanogaster*. Zschr. indukt. Abstamm. *86*: 439–458.

STERN, C., and E. W. SCHAEFFER. 1943. On wild-type iso-alleles in *Drosophila melanogaster*. Proc. Nat. Acad. Sc. U.S. *29*: 361–367.

TANTAWY, A. O. 1957. Genetic variance of random-inbred lines of *Drosophila melanogaster* in relation to coefficients of inbreeding. Genetics *42*: 121–136.

TANTAWY, A. O., and E. C. R. REEVE. 1956. Studies in quantitative inheritance. IX. The effects of inbreeding at different rates in *Drosophila melanogaster*. Zschr. indukt. Abstamm. *87*: 648–667.

VETUKHIV, M. 1952. Viability of hybrids between local populations of *Drosophila pseudoobscura*. Proc. Nat. Acad. Sc. U.S. *39*: 30–34.

——— 1954. Integration of the genotype in local populations of three species of *Drosophila*. Evolution *8*: 241–251.

——— 1956. Fecundity of hybrids between geographic populations of *Drosophila pseudoobscura*. Evolution *10*:139–146.

WALLACE, B. 1955. Inter-population hybrids in *Drosophila melanogaster*. Evolution *9*: 302–316.

——— 1956. Studies on irradiated populations of *Drosophila melanogaster*. J. Genet., Cambr. *54*: 280–293.

——— 1958. The average effect of radiation-induced mutations on viability in *Drosophila melanogaster*. Evolution *12*: 532–552.

WALLACE, B., and M. VETUKHIV. 1955. Adaptive organization of the gene pools of *Drosophila* populations. Sympos. Quant. Biol. *20*: 303–310.

WHITE, M. J. D. 1954. Animal Cytology and Evolution. 2nd revised edition. London and New York: Cambridge University Press.

THE MESOPHRAGMATICA GROUP AS AN EXAMPLE OF THE SPECIATION PHENOMENA IN *DROSOPHILA*

Danko Brncic[1]

SPECIES are Mendelian populations which possess a characteristic genetic structure. According to the definitions of Dobzhansky (1937) and Mayr (1942), a species is "that stage of the evolutionary process at which groups of actually or potentially interbreeding natural populations become reproductively isolated from other such groups." With some exceptions, this definition is applicable to sexually reproducing organisms, and it describes the basic process underlying the formation of species. Simpson (1951) has pointed out that a species or "a phyletic lineage (ancestral-descendant sequence of interbreeding populations) evolving independently of others, with its own separate and unitary evolutionary role and tendencies, is a basic unit in evolution."

Each living organism must be adapted to live in some environment. This adaptation is a result of a continuous process of natural selection which gives to the best-fitted genotypes more chance of perpetuation. To avoid extinction the organisms must not only be integrated in their actual environment, but also possess the ability to transmit to their offspring the capacity of adaptation to new environmental conditions. This capacity depends not on one or a few genes from determinate genotypes, but on the genetic structure of the population as a whole.

The process of speciation begins with the acquisition, by a group of populations, of a new genetic endowment which is adjusted to a certain new environment. But the process is not completed unless this new level of genetic integration is preserved by some mechanisms which prevent breakdowns of the new genetic endowments owing to intercrosses with neighboring populations. In other words, the development of reproductive isolating mechanisms is needed to preserve the evolutionary divergence. These two components of the process of speciation do not always proceed hand in hand since the acquisition of a new genetic structure may not be followed immediately by the development of reproductive isolation. When this happens, the evolutionary divergence stops at the subspecies level. On the other hand, reproductive isolation alone need not always lead to the emergence of new forms when it arises among different individuals of a given population. Summing up, both components are equally important for species formation, and broadly speaking depend on the same factors.

Nevertheless, the evolutionary events which take place depend upon the particular constellations of the evolutionary agents which act on populations of a given species or a group of related species. This is why comparative studies on the evolutionary patterns in different organisms are needed. There is as much reason to study comparative evolution as to study comparative anatomy. Interest in such comparative studies is clearly on the increase in population genetics. Bacteria, drosophila, rodents, and man, all evolve because of the interaction of mutation, natural selection, gene recombination, and genetic drift. But they evolve in different ways, and it is the business of the evolutionary geneticist to understand those differences.

[1]Cátedra de Biología, Escuela de Medicina, Universidad de Chile, Santiago, Chile.

According to Patterson and Stone (1952), the genus *Drosophila* had, in 1949, some 613 described species, and this number has been greatly enlarged since then. Although these species resemble each other rather closely in morphology, nevertheless it is clear that the genus *Drosophila* comprises forms with strongly contrasting population structures. There are nearly cosmopolitan forms such as *D. melanogaster*, *D. funebris*, *D. immigrans*, *D. hydei*, and others, whose population structures and ecology are strongly influenced by human activities. There are other species adjusted to "wild" habitats, and their distributional areas are confined to some definite territories. Some of these species live in vast and ecologically diversified territories without pronounced geographic barriers; the gene flow is possible between all the populations. Examples of such species are *D. pseudoobscura*, *D. willistoni*, and *D. robusta*. Other species are distributed discontinuously, in territories with many geographic or ecological barriers which prevent gene flow. Some of these are delicately adjusted to rare and specialized environments. Many of the non-domestic forms of the *repleta* group and the *virilis* group seem to be such species. It is probable that the members of the *mesophragmatica* group have many of the properties of such endemic species. These contrasting types of breeding structure of populations have a number of important consequences on speciation. Some of them will be discussed in the present paper.

Species of the *Mesophragmatica* Group and their Geographic Distribution

Six neotropical species of the subgenus *Drosophila* have been included by Brncic and Koref (1957a, b) in the *mesophragmatica* group: *D. mesophragmatica* (Duda, 1927); *D. gaucha* (Jaeger y Salzano, 1953); *D. pavani* (Brncic, 1957); *D. altiplanica* (Brncic and Koref, 1957); *D. orkui* (Brncic and Koref, 1957); and *D. viracochi* (Brncic and Koref, 1957). With the exceptions of *D. gaucha* and *D. pavani* which exhibit only cryptic morphological differences, all other members can be distinguished fairly easily by their external characteristics.

D. pavani have been found in Chile and in Argentina along the eastern slope of the Andes. Up to now, this species has not been collected north of Copiapó, Chile (27° 20′ S.), south of Valdivia, Chile (39° 52′ S.), or east of San Luis, Argentina (about 66° W.), where it overlaps the distribution area of *D. gaucha*. In the north-central part of Chile, *D. pavani* is the most abundant "wild" species of the genus, and its frequency in collections ranges between 7.85 per cent and 75.53 per cent, of the total number of individuals of all species of *Drosophila*.

D. gaucha seems to be geographically the most widely distributed species of the group. It has been found in southern Brazil, Uruguay, Argentina, and Bolivia. According to personal communications of Professor C. Pavan of the University of São Paulo, Brazil, and of Professor A. Cordeiro of the University of Rio Grande do Sul, Brazil, *D. gaucha* is, however, a very rare species in tropical Brazil. In Argentina it seems to be more abundant. In the "Sierra" of Cordoba (Argentina) it makes up 31.8 per cent, and in the "Sierra" of San Luis (Argentina) 16.8 per cent of the individuals of all species of the genus collected.

The data on the distribution and abundance of *D. mesophragmatica* are somewhat scant. We have enough information to be assured that the species is abundant in Bolivia and in Peru, but the species seems to be distributed farthest north along the Andes. Professor Dobzhansky has recently sent us a stock collected in mountains above Medellin, Colombia. In some collecting localities near Cuzco, Peru, the three species, *D. mesophragmatica*, *D. orkui*, and *D. viracochi* make up 51.35 per cent to 95.23 per cent of the total *Drosophila* population. Unfortunately, we have no data on the relative frequencies of the three members of the group.

D. orkui is sympatric with *D. mesophragmatica* and *D. viracochi* in Cuzco (Peru), and it has been collected also at Machu-Pichu (Peru). According to a personal communication from Professor M. Wheeler of the University of Texas, specimens very similar to *D. orkui* have been found by Dr. W. B. Heed in the mountains near Bogota, Colombia.

D. altiplanica was found only near La Paz (Bolivia) but, judging from the samples collected there, it is a very abundant species. Dr. W. B. Heed has collected some flies very similar to *D. altiplanica* in Colombia, near Bogota, at about 11,000 feet of altitude (personal communication of Professor M. Wheeler). *D. viracochi* has been collected only in Peru (Cuzco and Machu-Pichu) and we have no reliable information about its abundance in nature.

With the exception of *D. gaucha*, most of the species of this group seem to be fundamentally Andean. Its members are abundant in nature, and in some places they are the dominant species of *Drosophila*.

Levels of Evolutionary Divergence in the *Mesophragmatica* Group

The Level of Morphological Differences

Description and evaluation of morphological differences represent the oldest method of assessing the evolutionary status of a group of related species. Most often, species differ from each other in external traits, which are generally supposed to reflect the magnitude of the biological differences. No matter how important this criterion may be, there are exceptions to the rule: for instance, some species of the *obscura* group and of the *willistoni* group are morphologically almost indistinguishable. Nevertheless, there is a general agreement that these species are distinct biological entities; they do not interchange genes, and therefore follow separate evolutionary courses. From the biological standpoint, they constitute "good" species; the acquisition of reproductive isolating mechanisms makes the genetic interchange between different populations difficult or wholly impossible. As Dobzhansky (1955a) has pointed out, "the important thing is that when the process of speciation is completed, that is to say, when the reproductive isolating mechanisms have become finally established, the evolutionary divergence is irreversible." The existence of morphologically indistinguishable species ("cryptic" or "sibling" species) proves that morphological differentiation does not necessarily accompany genetic divergence (Dobzhansky, 1951, 1956). The sibling species are most important since they represent test cases of the distinction between races and species.

D. gaucha and *D. pavani* are, at present, indistinguishable by inspection of external traits. Not even a careful analysis of the male genitalia made at our laboratory by Dr. J. Nacrur could reveal differences that would permit separation of these species. Nevertheless, the lack of diagnostic differences does not mean that no differences exist. A statistical analysis of some of the common indices used in drosophila systematics, such as the wing indices, has shown that quantitative differences do exist between these two species. The same difficulties, though not so pronounced, arise when *D. altiplanica* and *D. orkui* are considered. The other members of the group, though rather similar externally, exhibit contrasting traits sufficient to permit recognition.

In spite of the fact that the members of the *mesophragmatica* group have so many common characteristics that some of them cannot be easily distinguished from one another, we are undoubtedly dealing with good biological species: they constitute closed genetic systems, each one following its own evolutionary path; they have different ecological requirements and distinct distribution areas. The reasons for

such scant morphological differentiation are obscure. Dobzhansky (1956) has suggested that the external morphology of some species groups of *Drosophila* has reached so high a level of adaptive specialization that most changes in the visible body structure are discouraged by natural selection; nevertheless, room is left for ecological divergence based on physiological differentiation.

The Reproductive Isolation Level

Brncic and Koref (1957b) and Koref, Casanova, and Brncic (1958) have studied some of the mechanisms responsible for reproductive isolation between the members of the *mesophragmatica* group. Table I shows that only six out of the thirty possible interspecific crosses yielded progeny. In the matings between *D. altiplanica* and *D. orkui* a few F_1 individuals emerged. The hybrid males had diminutive testes and were fully sterile. However, the females had normal gonads, and some of them gave offspring when backcrossed to either *D. altiplanica* or *D. orkui* males. In the crosses of *D. mesophragmatica* females with *D. pavani* or *D. gaucha* males, the few hybrids produced developed only to the pupal stage.

TABLE I

PRODUCTION OR NON-PRODUCTION OF HYBRIDS IN VARIOUS CROSSES BETWEEN MEMBERS OF THE *Mesophragmatica* GROUP

	Females					
Males	*meso-phragmatica*	*gaucha*	*pavani*	*orkui*	*altiplanica*	*viracochi*
mesophrag-matica	Yes	None	Few pupae	None	None	None
gaucha	Few pupae	Yes	Some ♂♂ and ♀♀ occasionally fertile	None	None	None
pavani	Few pupae	Sterile ♂♂ and ♀♀	Yes	None	None	None
orkui	None	None	None	Yes	Few sterile ♂♂ and some ♀♀ occasionally fertile	None
altiplanica	None	None	None	Few sterile ♂♂ and some ♀♀ occasionally fertile	Yes	None
viracochi	None	None	None	None	None	Yes

It is suggestive that in those matings in which no offspring were obtained, some of the females had no motile sperm in their genital tract. Since copulation was observed between the members of the six species, it seems clear that in those cases in which no progenies were produced, the heterospecific spermatozoa were quickly inactivated within the genital tract of the female. These observations resemble some of the findings of Patterson (1954) in crosses between members of the *virilis* group. However, the insemination reaction (Patterson and Stone, 1952) found in the *virilis* and other *Drosophila* species groups has not been observed in the *mesophragmatica* complex.

As hybrid males as well as females from the crosses between the sibling species *D. gaucha* and *D. pavani* apparently have normal gonads, they were tested for

TABLE II

HYBRIDS BETWEEN *D. gaucha* AND *D. pavani**

Crosses	P × P	$F_1 \times F_1$	♀ F_1 Backcrosses		♂ F_1 Backcrosses	
			With ♂ *gaucha*	With ♂ *pavani*	With ♀ *gaucha*	With ♀ *pavani*
♀ *gaucha* × ♂ *pavani*						
No. of crosses	50	318	33	36	30	30
Percentage with progeny	76.00	0	0	0	0	0
♀ *pavani* × ♂ *gaucha*						
No. of crosses	88	110	37	31	30	32
Percentage with progeny	79.54	3.63	24.32	0	20.00	9.37

*From S. Koref, A. Casanova, and D. Brncic (1958).

fertility. The F_1 females were crossed to F_1 males, and in another series of experiments, F_1 males and females were backcrossed to *D. gaucha* and *D. pavani*. Table II summarizes some of the results obtained. It can be observed that both male and female hybrids from matings of *gaucha* females with *pavani* males are completely sterile. On the other hand, some male and female progeny from the reciprocal cross, *pavani* females with *gaucha* males, were fertile, and a few flies were produced in all the backcrosses excepting those to *pavani* males. It is interesting to point out that no sperm or only non-motile sperm is found in the hybrid females from crosses of *D. gaucha* females to *D. pavani* males.

This difference between the reciprocal crosses cannot be explained by chromosomal differences since the hybrid females from both reciprocal matings have the same chromosomes from *D. pavani* and from *D. gaucha*. This suggests the existence of some cytoplasmatic or maternal effect modifying the degree of the reproductive isolation between these two species (cf. Patterson and Stone, 1952).

In all the matings, excepting those between *D. gaucha* and *D. pavani*, insufficient insemination and gamete mortality were found (Koref *et al.*, 1958). Moreover, in the crosses between *D. mesophragmatica* females and *D. gaucha* or *D. pavani* males, there was an early death of the hybrid zygotes which developed only to the pupal stage. The mortality of hybrid zygotes may be due to several causes, all related to genetic incompatibility. Cytological analysis of the salivary gland chromosomes of hybrid larvae (Brncic and Koref, 1957b) has shown that the species differ from one another in many inversions. Some sections of the chromosomes show no pairing, despite the fact that no clear-cut differences in the arrangement of the bands can be detected.

In the matings between *D. orkui* and *D. altiplanica* the above factors, and also hybrid sterility of the males, have been observed. The sterility is undoubtedly a consequence of the genetic incompatibility as the hybrid chromosomes exhibit a great number of inversions.

The two sibling species, *D. gaucha* and *D. pavani*, form viable hybrids which copulate normally, but their sperm in most cases is inactive. The exceptions observed refer only to a few cases in which *D. pavani* was the female parent. Both *D. pavani* and *D. gaucha* males when crossed with *D. mesophragmatica* females produced a few non-viable pupae and gave no progeny with the three other members of the group. These results, together with the similarities of the external characteristics, and of the chromosome configurations (Brncic and Koref, 1957b), further suggest that these two species are very closely related, and also are more closely related to *D. mesophragmatica* than to the other members of the group.

The absence of hybrids in nature in areas where both *D. pavani* and *D. gaucha* are sympatric is due probably to sexual (psychological) isolation. Data of Brncic and Casanova (Table III) indicate clearly that these crytic species have strong preferences for mating with representatives of their own species.

TABLE III

NUMBER OF FEMALES DISSECTED (*n*) AND PERCENTAGE INSEMINATED (%) AT DIFFERENT HOURS, IN CROSSES BETWEEN *D. pavani* AND *D. gaucha* IF MALES HAD THE CHOICE OF FEMALES OF BOTH SPECIES

		Homogamic		Heterogamic	
Males	Hours	*n*	%	*n*	%
D. gaucha	4	72	65.27	75	10.66
	12	92	64.13	99	25.25
	20	46	76.08	46	56.52
	24	49	100.00	50	98.00
D. pavani	4	67	25.37	70	7.14
	12	50	52.00	50	44.00
	20	48	79.00	47	57.44
	24	53	98.11	50	96.00

The Chromosomal and Genetic Difference Levels

Chromosomal reconstructions may be used as a tool for analysis of the phylogenetic relations of groups of species of *Drosophila*. Dobzhansky (1944) has pointed out that the probability of recurrence of exactly the same chromosomal inversion is very small; the inversions found in the populations are almost certainly results of unique events in the evolutionary history of a species. Using this hypothesis, phylogenetic schemes have been built in the *obscura* group (Dobzhansky, 1951), *virilis* group (Hsu, 1952), *repleta* group (Wasserman, 1954; Wasserman and Wilson, 1957), and others.

Pavan and Da Cunha (1947) and Jaeger and Salzano (1953) have studied the karyotype of *D. gaucha*, and Brncic and Koref (1957a, 1957b) the chromosomes of the other five species included in the *mesophragmatica* group. *D. pavani*, *D. gaucha*, and *D. viracochi* all have one pair of V-shaped chromosomes, three pairs of rods, one of which is the X-Y pair, and one pair of dots. *D. altiplanica* has the same basic chromosomal configuration although its dots are slightly elongated, and one of the pairs of rods is bent, appearing as small V's. *D. mesophragmatica* and *D. orkui* both have one pair of V's and four pairs of rods. One of the pairs of rods is shorter than the others and corresponds to the dots observed in the other species which has been modified through the addition of extra heterochromatin. Despite the differences in the metaphase, the salivary gland chromosomes appear to be at first glance very similar in all six species. There are five long euchromatic arms which converge towards a large heterochromatic chromocenter, in which is embedded a small element, corresponding to the dot-like chromosome. The species can, however, be identified cytologically by observing the disk patterns of the corresponding chromosomes (Brncic and Koref, 1957b).

Precise comparison of the gene arrangements in the members of the *mesophragmatica* group is made difficult by the failure of most of them to produce hybrids. However, some phylogenetic inferences can be made on the basis of the comparative analysis of the metaphase plates, the salivary gland cells of the few

hybrids obtained, morphological differences, and the degree of reproductive isolation. The cryptic species, *D. gaucha* and *D. pavani*, seem to be most clearly related. Their metaphase plates are identical, but a study of the giant chromosomes of the hybrid larvae shows a double overlapping inversion in the basal part of the sex chromosome. Because of these inversions, a large heterochromatic zone in the X-chromosome which is submedian in *D. pavani* is sub-basal in *D. gaucha*. This condition, easily visible, is diagnostic for the distinction of *D. pavani* both from *D. gaucha* and from the other four species (Brncic and Koref, 1957b).

D. viracochi has the heterochromatic region of the X-chromosome in a sub-basal position. On this basis, the last-named species is considered to be closer to *D. gaucha* than to *D. pavani* and it is placed accordingly in Figure 1. *D. mesophragmatica* and *D. orkui* are probably related since they have identical metaphase configurations, although they do not mate, and differ slightly in their external ap-

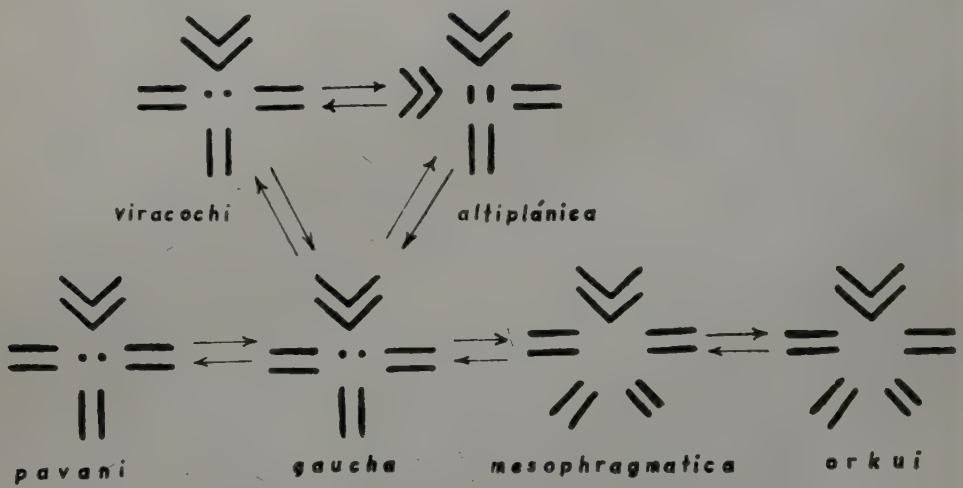


FIGURE 1. Metaphase plates and hypothetical phylogenetic relations of the six members of the *mesophragmatica* group (from Brncic and Koref, 1957b).

pearance. Since the crosses of *D. mesophragmatica* with *D. gaucha* and *D. pavini* produce a few hybrids which die in the pupal stage, it may be conjectured that these species are closer to each other than they are to *D. orkui*. Every chromosome of *D. mesophragmatica* differs from the homologous ones of *D. pavani* or *D. gaucha* by mutiple inversions, as well as by lack of pairing of certain regions, apparently owing to a lack of homology between some of the bands. These findings seem to indicate that these species have diverged considerably.

Although the metaphase plate of *D. altiplanica* places it near to *D. gaucha* and to *D. viracochi* (Brncic and Koref, 1957b), its external characteristics and the degree of reproductive isolation bring it nearer to *D. orkui*. This makes it difficult to establish the phylogenetic position of *D. altiplanica* with certainty.

Comparison of the chromosomes of the six species has revealed that the speciation has been accompanied by paracentric inversions and by additions or by losses of heterochromatin. This does not imply that mutational changes and rearrangements at a lower chromosomal level have not taken place as well.

Polymorphism and Population Structure

Many species of *Drosophila* exhibit a special type of polymorphism due to inversion of blocks of genes in their chromosomes. A large amount of experimental data in the laboratory, and in nature, has demonstrated that this polymorphism is adaptive and balanced (cf. Dobzhansky, 1951, and others). The heterozygotes which carry two chromosomes of a pair with different gene arrangements are generally heterotic since they possess a higher adaptive value than the homozygotes, in at least some of the environments which the populations containing them normally encounter. However, the intraspecific variability may take different forms, and chromosomal inversions are characteristic of some species but not of other. Even within a group of species having variable gene arrangements, some species have more inversions than other. A comparative study of groups of closely related species of *Drosophila* has permitted Dobzhansky *et al.* (1950) and Da Cunha *et al.* (1953) to establish interesting relations between the degree of chromosomal polymorphism and the capacity of a species to exploit the ecological opportunities offered by its environment.

Some data bearing on the population structure in species of the *mesophragmatica* group are available (Brncic, 1957b, 1957c). The structure of the salivary gland chromosomes, as indicated above, is much alike in all members of the group. Brncic (1957b) has published the "standard" salivary gland chromosome map of one of the species, *D. pavani*. The disk patterns are sufficiently similar in the homologous chromosomes of the six species under consideration, thus permitting an easy recognition of these chromosomes. The observed variations in the gene sequences can than be referred to the "standard" map of *D. pavani*.

Table IV summarizes the data of the number of variants in the gene orders found within each of the six members of the *mesophragmatica* group. *D. pavani* has eight inversions, *D. orkui* exhibits five, and *D. mesophragmatica* four. In the other

TABLE IV

Comparison of the Number of Different Inversions Found in the Chromosomes of Natural Populations of the Members of the *mesophragmatica* Group*

Species	Chromosomes					
	I	II	III	IV-R	IV-L	Total
D. pavani	–	2	–	3	3	8
D. gaucha	–	–	–	–	–	0
D. mesophragmatica	–	–	1	–	3	4
D. orkui	–	3	2	–	–	5
D. altiplanica	–	–	–	–	–	0
D. viracochi	–	–	–	–	–	0
Total	0	5	3	3	6	17

*From Brncic (1957c).

three species no variations have been found. It can be concluded that with the exception of the first chromosome (the sex pair), inversions have been found in all the large chromosomes. In *D. pavani* the inversions are concentrated in the second chromosome and in both arms of the fourth chromosome. In the second chromosome two inversions have been discovered, one of them included in the other. These two inversions have never been found separately; both were present in practically

all Chilean and Argentinian populations studied. The frequency of heterozygotes for these inversions ranges from 28 to 56 per cent in the different populations. In all samples analyzed there were found also three overlapping inversions in the right limb of the fourth chromosome; the heterozygotes for this condition occur in frequencies which range from 46 to 60 per cent in different populations. Finally, in the left arm of this same chromosome, most populations studied contained heterozygotes of another complex gene arrangement which differs from "Standard" by three overlapping inversions. The frequency of heterozygotes for this gene sequence ranges from 38 to 62 per cent. In none of the populations, and in none of the crosses between them, have these inversions in the right arm or those in the left arm been found isolated; they always occur together as complexes.

In *D. mesophragmatica*, inversions were found in the third and in the left arm of the fourth chromosome (Brncic, 1957c). In the third chromosome a small inversion was present in heterozygous condition in 10 to 20 per cent of the strains from Bolivia and Peru. In the left limb of the fourth chromosome, more than 50 per cent of all larvae examined were heterozygotes for a complex of three small inversions. None of these populations showed any of the three inversions separate from the other. The only population of *D. orkui* studied was heterozygous for three inversions in the second chromosome and for two inversions in the third.

The occurrence in nature of complexes of inversions and the apparent lack of separate occurrence of the constituents of these complexes are interesting. The number of inversions which must have occurred during the evolutionary development of species must have been rather large. It is possible that the intermediates between the gene arrangements found in natural populations of *D. pavani* and *D. mesophragmatica* will be discovered when more and larger samples can be examined. But even in this case it would remain true that the connecting links will be rare in each species as a whole. The hypothesis of Wallace (1953) provides a possible explanation for the absence in natural populations of the gene arrangements intermediate between the existing terminal links of the inversions series. According to Wallace, the adaptive role of inversions is to maintain intact coadapted complexes of polygenes which give rise to heterosis in heterozygotes. This coadaptation might be lost if gene exchange were to occur breaking up the coadapted gene complexes. Such a situation is likely to occur if three gene arrangements representing the three links in the chain of overlapping inversions (the "triads" of Wallace) coexisted in the same population. Crossing-over then might result in transfer of blocks of genes from one chromosome with one gene arrangement to another chromosome with a different arrangement, and this might lead to disintegration of the coadapted gene systems. As Wallace has pointed out, this mechanism might explain the fact that in most populations of *D. pseudoobscura* only two members of the "triads" are found with high frequencies. The selective pressures which control the frequency and the distribution of the chromosomal forms would, according to Wallace, maintain the adaptive gene combinations guarded by the inversions. This may explain the absence of the intermediate gene arrangements between the "standard" and the chromosomal types found in nature in *D. pavani* and *D. mesophragmatica.*

In addition to the absence of intermediate steps between the gene arrangements actually observed in nature, the adaptive importance of the chromosomal polymorphism present in the populations of members of the *mesophragmatica* group can be inferred in several ways. The most significant fact is the high incidence of inversion heterozygotes found in *D. pavani* and *D. mesophragmatica*. Some inver-

sions in the heterozygous condition exceed a frequency of 50 per cent in most populations. This would not be likely to occur if both the homozygotes and the heterozygotes were equally viable. There appears to be differential mortality favoring the heterozygotes in at least some populations.

Population Structure and Patterns of Speciation

D. pseudoobscura (Dobzhansky, 1951), *D. robusta* (Carson and Stalker, 1947), and *D. willistoni* (Da Cunha *et al.*, 1950; Da Cunha and Dobzhansky, 1954) are outstanding examples of species not associated with man, which nevertheless have very wide areas of distribution and are very abundant in nature. Contrasting with them, most of the non-domestic forms on the *repleta* group, the *virilis* group, and the six members of the *mesophragmatica* group are typically endemic species living in strongly isolated territories. The species of the *repleta* group in North America seem to be specialized to occupy the few adaptive niches offered by the semi-desert and desert regions. The species included in the *mesophragmatica* group, with the exception of *D. gaucha*, are fundamentally Andean. The configuration of the Andean mountain system is especially favorable for the formation of local isolated populations. *D. pavani* lives in the north-central part of Chile and in Argentina close to the eastern slope of the Andes. *D. gaucha* is rather abundant only in Argentina and seems to be scarce in southern Brazil and Bolivia. *D. mesophragmatica* was found only in the high zones of Colombia, Peru, and Bolivia. *D. altiplanica* was found only in Bolivia. Finally, *D. orkui* and *D. viracochi* have been collected only in Peru.

Dobzhansky *et al.* (1950), Da Cunha *et al.* (1953), and Da Cunha and Dobzhansky (1954) inferred that a more restricted species is likely to be ecologically less versatile, and therefore may be expected to be less polymorphic in comparison to a related but more versatile and wide-ranging species. Species as widely distributed as *D. willistoni* may be expected to have a high frequency of heterozygotes for inversions. In contrast to this, members of the *repleta* group (Wasserman, 1954, 1957) or of the *mesophragmatica* group (Brncic, 1957b, 1957c) are less polymorphic. The only geographically rather widespread species of the *mesophragmatica* complex, *D. gaucha*, is very rare in the major part of its area. The amount of chromosomal polymorphism may be correlated with the complexity of the habitats.

Such contrasting types of population structures could be explained in connection with the adaptive role of the chromosomal polymorphism due to inversions. Since inverted sections in the chromosomes reduce the recombination by crossing-over, groups of selected polygenes may be maintained free from disruption. In nature, such coadapted gene systems are preserved by the superior fitness of the heterozygotes which carry two chromosomes of a pair with different gene arrangements. Put in another way, chromosomal inversions are inherited as single units and owing to their heterotic condition are preserved in natural populations.

Carson (1955a, 1955b) and Stone (1955) have pointed out that the accumulation of inversion polymorphism may result in reduced ability of the population to evolve new adaptive gene combinations. New combinations may, however, arise by recombination of the linked series of the polygenes maintained in the inverted zones of a chromosome. High polymorphism is observed in species that live in regions characterized by many and rich ecological niches, in which populations of tremendous sizes can develop. Too much polymorphism seems to be unfavorable in

species adjusted to ecologically less versatile areas. The desert species, as Stone has pointed out (1955), including many of the *repleta* group, seldom have extensive chromosomal polymorphisms. Free recombination seems to be more advantageous in these smaller populations. However, high potentials for recombination can be observed in the marginal populations of wide-ranging species. In *D. robusta* (Carson, 1955a, 1955b), and in *D. willistoni* (Da Cunha and Dobzhansky, 1954), marginal populations are cytologically less polymorphic. The peripheral populations of wide-ranging species, small populations of species found in the desert conditions, on isolated islands, and species with discontinuous range on mountainous territories are genetically more flexible according to the above views. This situation leads to the development of many different forms and to a rapid race and species formation (Mayr, 1954).

There are also other differences between endemic and wide-ranging species. In widely distributed species, populations usually exhibit variations arranged in graded series or clines in correspondence with the environmental gradients (Rensch, 1939; Huxley, 1939; Mayr, 1942; Dobzhansky, 1951; Carson, 1955b). These adaptive responses to the environmental differences very seldom give rise to new species, owing to the gene flow between the neighboring populations. Examples of this sort have been found not only in *Drosophila* but also in many other animals (Rensch, 1939; Huxley, 1939; Mayr, 1954).

The situation is quite different in more restricted species. They must be adapted to a rigorous environment which normally does not exhibit clinal variations. Moreover, the gene exchange in a more restricted area of distribution contributes to the maintenance of more uniform gene or chromosomal frequencies. Owing to the clinal variation observed in widely distributed species, there are no missing links between the extreme grades of variations. In contrast, such links as the intermediary steps in a chain of overlapping inversions observed in many species of *Drosophila* should be rapidly eliminated by the selective factors of the environment if they are actually non-adaptive.

The above arguments seem to be adequate to explain the absence of most of the gene sequences intermediate between those found in the natural populations of *D. pavani* and *D. mesophragmatica*. Though we are far from knowing the exact area of geographic distribution of these two forms, we have enough information to show that they are geographically rather restricted species. They are strongly heterozygous for a few gene orders occurring in the entire known range of their distribution. The balanced polymorphic condition maintained by these inversions could be accounted for by the fact that these species form flourishing populations and many of them represent the dominant forms in their regions. On the other hand, the absence of the intermediate links between the chromosomal arrangements found in nature could be the consequence of their non-adaptive nature. The genetic and chromosomal structure of a species is the result of natural selection. Species or popualtions adjusted to a discontinuous territory tend to diverge more rapidly. The common ancestors of the six members of the *mesophragmatica* group, being transformed into isolated endemic species in the various places of the Andes, have acquired by the selective pressures the gene structure best adjusted to each region (Brncic, 1957c). Stone (1955) has already pointed out that "the *repleta* group, having achieved the ancestral genetic system which made desert adaptation possible, has evolved into the largest species group in *Drosophila* with over fifty species."

We have attempted to show that isolated endemic species differ from widely distributed species with respect to the genetic structure of the populations. The

differences observed have a number of important consequences for the interpretation of the evolutionary patterns. According to most evolutionists, species can evolve in the following two ways: by splitting of a population into two or more descendant groups, and by transformation of a species as a whole without multiplication of the number of forms.

Closely related species of *Drosophila*, such as those belonging to the *repleta* group or to the *mesophragmatica* group, which are narrowly adapted to separate zones of a discontinuous territory, may represent the splitting of a single ancestral population into sharp races, some becoming differentiated into good biological species by the development of reproductive isolating mechanisms. Widespread species in a continuous territory can evolve as single units.

It has already been said that wide-ranging species of *Drosophila*, such as *D. pseudoobscura*, *D. robusta*, and *D. willistoni*, show clinal variation of many characters such as certain chromosomal inversions, but other genetic variants are present in practically all the range of the geographic distribution of the species. As a general rule these variants which are so extended are adaptively important. Two hypotheses could explain the origin of these highly useful genotypes. One is that they constituted very old acquisitions that have reached their actual distribution following the spreading of the species. On the other hand, Dobzhansky has emphasized that those omnipresent variants could be originated in any one population and that, owing to their superior adaptive nature, they could extend over the entire area of distribution of the species. This means that the genetic constitution of the actually living species is a result of the sum of the favorable genetic improvements which occurred at different places and different times, allowing the species as a unit to reach a superior adaptive level. In other words, the evolutionary paths of at least some species do not consist in the substitution of the whole species by those populations which have acquired a better level of adaptation. Natural selection does not seem to favor such "superior" races in the conquest of the ecological niches already occupied, displacing the other communities of the species. Natural selection could act favoring the spread of the better suited genetic improvements originated in any one population by means of intercrossing with the neighboring populations.

The data of distribution of some characters, as chromosomal inversions, in wide-ranging species such as *D. willistoni*, seem to give good support for that point of view. In that form and in other widespread forms, certain inversions are present in the whole species. If each inversion has arisen only once, they may expand over enormous distances and territories impelled by natural selection. If that is the case, a species such as *D. willistoni* evolves as an evolutionary system, incorporating favorable mutants and gene combinations even if these arise in remote parts.

It is important to keep in mind that such a model of evolution seems to be closely comparable with that already postulated for the human species. At present, there is no good evidence for the existence in the past of more than a single human or pre-human species. Modern *Homo* has resulted from the intermarrying of many different human or pre-human communities, and each one has contributed its best genes. As Dobzhansky has stressed (1955), "the gene pool of the now living mankind contains genetic elements which were present in many and perhaps in all major populations of the past."

The discovery of the actual genetic differences among related species or subspecies is very important, but it represents only the first step in the study of evolutionary divergence. For the development of new forms it is necessary that the genetic systems acquired by the different populations should not be lost by inter-

breeding. Put in another way, speciation is completed only with the emergence of reproductive isolating mechanisms.

We have already analyzed some of the isolating mechanisms which prevent gene exchange between the different members of the *mesophragmatica* group (Brncic and Koref, 1957; Koref *et al.*, 1958). Nevertheless, we still know very little about the origin of such mechanisms. Muller (1949) has supposed that reproductive isolation may arise by chance as a by-product of other changes in the genetic systems. For instance, genes may arise by mutation, whose isolating effects may become evident only in combination with other genes. "So, it is necessary to conclude, that isolating genes were not established by a process of selection which derived its original advantage from the achievement of the isolating effect itself, but were established, in their original local populations at any rate, through their selective value in other respects, or through drift, as the case may be." On the other hand, Dobzhansky (1951) thinks that "Development of all forms of reproductive isolation, including sexual isolation, between populations which have diverged sufficiently, may be initiated and furthered by natural selection."

Both chance and selection are unquestionably important in the development of the reproductive isolating mechanisms. The alternative hypothesis of Dobzhansky has been demonstrated in several ways in the laboratory (cf. Dobzhansky, 1951, 1955b), and recently Koref and Waddington (1958) have shown that tendencies toward homogamic mating may arise by chance within inbred lines, thus giving a rather good support for the Muller hypothesis. In any case, it seems necessary, for the formation of genetic isolating systems, that the populations live in different territories. High adaptive levels for the species can only be maintained by those groups of local populations among which there is reproductive compatibility. Thus, selection should favor homogamic mating. In this respect, naturally isolated populations resemble the inbred strains maintained in a laboratory rather than selected lineages (Koref and Waddington, 1958).

Acknowledgments

The author is very much indebted to the University of Chile and to the Rockefeller Foundation, for the financial aid which made the present work possible. Thanks are also due to Professors Th. Dobzhansky and G. Hoecker, for their constructive criticism and help in the preparation of the manuscript; and to Miss Adriana Casanova for technical aid.

References

Brncic, D. 1957a. Las especies chilenas de Drosophilidae. Col. Monografías biológicas Univ. Chile, 8. Santiago: Universidad de Chile.

——— 1957b. Chromosomal polymorphism in natural populations of *Drosophila pavani*. Chromosoma *8*: 699–708.

——— 1957c. A comparative study of chromosomal variation in species of the *mesophragmatica* group of *Drosophila*. Genetics *42*: 798–805.

Brncic, D., and S. Koref Santibañez. 1957a. The *mesophragmatica* group of the genus *Drosophila* with description of three new species. Biológica (Chile) *22*: 63–72.

——— 1957b. The *mesophragmatica* group of species of *Drosophila*. Evolution *11*: 300–310.

Carson, H. L. 1955a. Variation in genetic recombination in natural populations. J. Cellul. Physiol. *45* (suppl. 2): 221–236.

——— 1955b. The genetic characteristics of marginal populations of *Drosophila*. Sympos. Quant. Biol. *20*: 276–287.

CARSON, H. L., and H. D. STALKER. 1947. Gene arrangements in natural populations of *Drosophila robusta* Sturtevant. Evolution *1*: 113–133.

DACUNHA, A. B., D. BRNCIC, and F. M. SALZANO. 1953. A comparative study of chromosomal polymorphism in certain South American species of *Drosophila*. Heredity *7*: 193–202.

DACUNHA, A. B., H. BURLA, and TH. DOBZHANSKY. 1950. Adaptive chromosomal polymorphism in *Drosophila willistoni*. Evolution, *4*: 212–235.

DACUNHA, A. B., and TH. DOBZHANSKY. 1954. A further study of chromosomal polymorphism in *Drosophila willistoni* in its relation to the environment. Evolution *8*: 119–134.

DOBZHANSKY, TH. 1937. Genetics and the origin of species. 1st. ed. New York: Columbia Univ. Press.

——— 1944. Chromosomal races in *Drosophila pseudoobscura* and *Drosophila persimilis*. Pub. Carnegie Inst. Washington. *554*: 47–144.

——— 1951. Genetics and the Origin of Species. 3rd revised ed. New York: Columbia Univ. Press.

——— 1955a. The genetic basis of systematic categories. *In* Proc. XVI Biol. Colloq., Oregon State College, "Biological Systematics," pp. 37–42.

——— 1955b. Evolution, Genetics, and Man. New York: John Wiley & Sons, Inc.

——— 1956. What is an adaptive trait? Am. Natur. *90*: 337–347.

DOBZHANSKY, TH., H. BURLA, and A. B. DACUNHA. 1950. A comparative study of chromosomal polymorphism in sibling species of the *willistoni* group of *Drosophila*. Am. Natur. *84*: 229–246.

HSU, T. C. 1952. Chromosomal variation and evolution in the *virilis* group of *Drosophila*. Univ. Texas Pub. *5204*: 35–72.

HUXLEY, J. 1939. "Clines": an auxiliary method in taxonomy. Bijdr. Dierk., *27*: 49.

JAEGER, C. P., and F. M. SALZANO. 1953. *Drosophila gaucha*, a new species from Brazil. Rev. brasil. biol. *13*: 205–208.

KOREF SANTIBAÑEZ, S., A. CASANOVA, and D. BRNCIC. 1958. Experiments on sexual isolation in species of the *mesophragmatica* group of *Drosophila* (Diptera). Proc. R. Entomol. Soc., Lond. *33*: 179–185.

KOREF SANTIBAÑEZ, S., and C. H. WADDINGTON. 1958. The origin of sexual isolation between different lines within a species. Evolution *12*: 485–493.

MAYR, E. 1942. Systematics and the Origin of Species from the Viewpoint of a Zoologist. New York: Columbia Univ. Press.

——— 1954. Changes of genetic environment and evolution. *In* Evolution as a Process, pp. 157–180. London: Allen & Unwin.

MULLER, H. J. 1949. The Darwinian and modern concepts of natural selection. Proc. Am. Philos. Soc. *93*: 459–470.

PATTERSON, J. T. 1954. Fate of the sperm in the reproductive tract of the Drosophila female in homogamic matings. Univ. Texas Pub. *5422*: 19–37.

PATTERSON, J. T., and W. S. STONE. 1952. Evolution in the Genus *Drosophila*. New York: Macmillan Co.

PAVAN, C., and A. B. DA CUNHA. 1947. Especies brasileiras de *Drosophila*. Bol. Fac. fil. cienc. e letr., Univ. S. Paulo, 86. Biol. geral., *7*: 3–46.

RENSCH, B. 1939. Klimatische Auslese von Grössenvarianten. Arch. Naturgesch., n.f. *8*: 89–129.

SIMPSON, G. G. 1951. The species concept. Evolution *5*: 285–298.

STONE, W. S. 1955. Genetic and chromosomal variability in *Drosophila*. Sympos. Quant. Biol. *20*: 256–270.

WALLACE, B. 1953. On coadaptation in *Drosophila*. Am. Natur. *87*: 343–358.

WASSERMAN, M. 1954. Cytological studies of the *repleta* group. Univ. Texas Pub. *5422*: 130–152.

WASSERMAN, M., and F. D. WILSON. 1957. Further studies on the *repleta* group. Univ. Texas Pub. *5721*: 132–156.

GENE SYSTEMS REGULATING CHARACTERS OF ECOLOGICAL RACES AND SUBSPECIES

Jens Clausen[1]

THE SUBJECT of genetics in evolution extends beyond the framework of pure genetics to a study of how products of genetic variation fit into environmental niches. Up to the present time genetics has by itself made little contribution to an understanding of how genes function in evolutionary processes. Such a study requires a combination of genetics with the branch of ecology that is concerned with the influence of the environment on the organism and the fitness of the organism to the environment. Geneticists are by nature interested in standardizing rather than diversifying the environment and, therefore, neglect studying the influence of varied environments on the phenotypic expression of the genotype. Ecologists neglect studying genes. Geneticists and ecologists have made frequent excursions into this no-man's-land of biology, but lack of solid information causes the speculations of both to appear fanciful.

There are several reasons why genetics has failed in clarifying the processes of evolution. In the first place, the genetic mechanisms governing variation of living things in the wild are poorly understood. Cultivated and laboratory organisms that are unrelated to natural habitats have been used in genetic experiments in preference to wild ones for very good experimental reasons.

Major emphasis has been placed upon individual genes rather than on the expression of characters regulated by systems of genes. Natural selection does not operate on the individual gene but on organisms that vary in their characters and combinations of characters. The influence of natural selection on the gene frequency in a population is, therefore, indirect rather than direct, a fact sometimes forgotten in discussions on selection.

Very limited knowledge is available about the degree to which a gene or a combination of genes can be expressed phenotypically over a range of environments. Latent genes are far more common than has generally been assumed, but this area has not been thoroughly explored. Latent genes are those that require specific environments for their activation, those that lack complementaries, and those that are inhibited by other genes.

Only differences between genes can be effectively studied. Most crossings have been between closely related organisms that have many genes in common. Crossings between contrasting ecotypes and subspecies within a species reveal genes that could not be discovered in a more restricted crossing program. The homozygous genetic core of a species is also unknown to us. This core is presumably the most significant element in the evolution of a species, but it ceases varying after the species becomes fully established.

A final important factor in the evolution of natural entities is the existence of coherence between the genes that regulate those groups of characters that fit the species to its range of environments. Coherence is attained in some organisms

[1]Department of Plant Biology, Carnegie Institution of Washington, Stanford, Calif., U.S.A.

through linkage of some of the genes that control groups of characters; in other organisms it is effected through inversion and translocation segments that bind genes together within the segment; in still other organisms coherence is achieved through differential selection. Some of the aspects of coherence in genetics are a little better understood than the other four aspects mentioned above.

In the following discussion we will limit ourselves primarily to the first three aspects: namely, the genetic structure of individuals from contrasting natural habitats, the gene systems that regulate individual characters of evolutionary significance, and the phenotypic expression of certain genes in diverse environments. Examples will be taken from plants that have relatively simple gene systems.

Gene Systems Regulating *Layia* Ecotypes

Layia platyglossa, the tidytip of the sunflower family, is a 7-chromosome spring annual of California lowlands and Coast Ranges. Its populations in northern and southern California are physically separated by the San Bernardino Mountains and a distance of 75 kilometers over which the species does not occur. In northern California the species has evolved a maritime ecotype along the wind-swept bluffs of the cool coast, an inland ecotype in the Coast Ranges, and a valley ecotype in the Central Valley. The maritime differs from the inland ecotype in lacking a central stem, in having horizontal side branches from the base, and in being late-blooming. The southern California populations differ from those north of the mountains in the expression of several characters (Clausen and Hiesey, 1958; Clausen, 1951). The coasts of southern California are milder, and the maritime ecotype is absent there.

Complications for genetic interpretation arise when more than a single hybrid of a kind are studied. Shifts in dominance occur, and ratios of segregation vary from hybrid to hybrid as in *Layia* (Table I). For example, presence of central stem is the recessive condition in the cross of Jenner $\times$ San Bernardino, but it is dominant in the cross Cambria $\times$ Pala. Dominance is also reversed in the inheritance of orientation of branching. Ratios such as 43 : 21 and 9 : 7 indicate the presence of complementary genes, whereas 13 : 3 suggests the interaction of genes having opposite effects. The genotypes are therefore not directly related to the phenotypes.

The inheritance of central stem is relatively simple. Its segregation suggests a system of only three pairs of genes. A positive gene, *S*, is basic for the presence of stem, but this gene can be suppressed by an inhibitor, *In*, which, in turn, requires the presence of a complementary gene, *K*.

Orientation of branching varies among the F_2 progenies between horizontal and erect, but the inheritance of this character is slightly more complicated than that of the central stem. The Pala plant from southern California represents the basic recessive form and has erect branches. The presence of two complementary genes, *H* and *C*, such as in the Cambria population on the coast, cause the branches to become horizontal. Finally, two epistatic genes, A_1 and A_2, of the inland Etiwanda population cause the branches to ascend again. There are, therefore, two kinds of erect branching, the hypostatic and the epistatic.

The maritime populations from Jenner and Point Joe belong to the same ecotype, although geographically they are 225 kilometers apart. The genes that determine their maritime characteristics are the same, for a cross between these two populations produced 1,400 F_2 plants, all of the maritime type, namely, without central

TABLE I

INHERITANCE OF ECOTYPIC DIFFERENCES IN *Layia platyglossa*

Parents						
MARITIME	INLAND	F_1	F_2			Total
CENTRAL STEM						
Absent	Present		Absent		Present	
Jenner	× San Bernardino	absent	852		459	1,321
*InKs**	*inkS*		43	:	21	
Point Joe	× Etiwanda	absent	476		327	803
InKS	*inkS*		9	:	7	
Cambria	× Pala	present	205		621	826
Inks	*inkS*		1	:	3	
BRANCHING						
Horizontal	Ascending		Horizontal		Ascending	
Jenner	× San Bernardino	ascending	218		1103	1,321
a_1a_2HC†	A_1a_2hC		3	:	13	
Point Joe	× Etiwanda	ascending	69		734	803
a_1a_2HC	A_1A_2HC		1	:	15	
Cambria	× Pala	horizontal	500		325	825
a_1a_2HC	a_1a_2hc		9	:	7	
EARLINESS						
Late	Early		Late		Early	
Jenner	× San Bernardino	early	205		1116	1,321
$e_1e_2e_3L$‡	$E_1e_2e_3l$		3	:	13	
Point Joe	× Etiwanda	early	16		787	803
$e_1e_2e_3L$	$E_1E_2E_3L$		1	:	63	
Cambria	× Pala	early	39		786	825
$e_1e_2e_3L$	$E_1E_2e_3L$		1	:	15	

**In* and *K*: epistatic complementary inhibitors of central stem; *S*: hypostatic gene for central stem; *ss* and *InKS*: central stem absent.

†A_1 and A_2: epistatic, multiple genes for ascending branches; *H* and *C*: hypostatic complementary genes for horizontal branching; a_1a_2hc: ascending-erect.

‡E_1, E_2, and E_3: epistatic multiple genes for early flowering; *L*: hypostatic gene for late flowering; $e_1e_2e_3l$: early flowering.

stem, having prostrate branches and being late-flowering. This appearance is in accordance with the gene formulae of the parental plants in Table I.

In the F_2's of the interecotypic crossings of *Layia*, the parental combinations of growth habit and of time of flowering occurred more frequently than expected if free recombination had taken place. Some of the genes that regulate the parental characters are therefore linked. Together with natural selection, such genetic cohesion keeps even neighboring ecotypes distinct under natural conditions.

Natural selection has operated for ages in the northern California coastal regions and has separated ecotypes of *Layia platyglossa* that are morphologically and physiologically distinct. Certain parallel gene combinations have been selected at widely separated points along the exposed coasts and others in the inland zones. The ecologically important functions of these ecotypes are regulated by distinctive systems of genes that have cumulative, opposite, or complementary effects. These systems are flexible, and they provide a balance between the perpetuating influences of genetic cohesion and the diversifying influences of moderate genetic recombination. If the environment changes, the balance shifts in favor of recombinations.

Date of Flowering in Ecotypes of *Layia* and *Madia*

The earliness, or the date of first flowers of a plant, is likewise regulated by genes of opposing effects. In *Layia platyglossa* (Table I) there are at least three pairs of genes, E_1 to E_3, that promote early flowering as seen in the inland ecotype, but these are epistatic over a gene L, that promotes lateness. The simplest segregation, 13 : 3, is found in the cross Jenner × San Bernardino. In the hybrid Point Joe × Etiwanda there was a frequency ratio of 63 early to 1 that bloomed as late as the Point Joe.

In *Madia elegans*, belonging to a taxonomically related genus, there is a similar system regulating the time of flowering, although the ecotypes of *Madia elegans* are adjusted to entirely different seasons of the year. *Madia elegans* is an 8-chromosome annual of California Coast Ranges and foothills in which spring- and fall-blooming ecotypes occur in the same kinds of places but are adjusted to different kinds of seasons. Both ecotypes germinate after the fall rains and continue slow growth during the mild winter. The spring ecotype develops stems in the cool spring, begins flowering in March to May, and completes its life cycle before summer heat and drought set in. The fall ecotype responds very differently to the same set of climatic conditions. During winter and spring it develops only dense basal leaf rosettes and no stems, but at a time when the spring form has died, the fall-blooming ecotype begins to develop towering, densely leafy, and highly glandular stems that reach the flowering stage two to three months later than the spring ecotype.

The two races were crossed artificially. The F_1 was morphologically intermediate, but was as early-blooming as the spring race. It was fully fertile, and an F_2 population of more than 1,100 plants was classified (Table II).

TABLE II

Inheritance in Seasonal Ecotypes of *Madia elegans*: Date of First Flowers*

Parents		F_1	F_2			
May	August	June 1	May 10–31	June 1–July 8	July 9–Aug. 1	Total
Vernal ×	Autumnal	vernal	796	323	16	1,135
			851.2	266.0	17.8	1,135.0
			48 :	15 :	1	
El_1l_2†		eL_1L_2†	$EeL_1l_1L_2l_2$	$E\ldots$	$eeL_1\ldots$	$eeL_1L_1L_2L_2$

*After Clausen and Hiesey, 1958.

†E: epistatic gene causing early flowering; L_1 and L_2: hypostatic cumulative genes causing late flowering.

Among 64 F_2 plants a ratio of 48 (three-fourths) flowered in May together with the spring ecotype, and a ratio of 15 flowered during June. Only one out of 64 flowered in July, and none in August when the fall-blooming parent reached maturity, indicating that the F_2 population was too small. A dominant, epistatic gene, E, determines, therefore, early blooming; this gene is epistatic over a series of at least two genes, L_1 and L_2, that promote late flowering. The plants that come into flower during June and July possess various numbers of the lateness genes but not the epistatic earliness gene.

In the F_2 there was also strong, although not absolute, correlation between late flowering, tall stems, and densely glandular pubescence, so that the parental characters cohered. The gene systems and correlations that control these *Madia* ecotypes

are such that they favor the sorting out of the parental forms. Swarms of hybrids like the one described could fairly quickly be expected to sort out again into spring and fall ecotypes if they were exposed to natural selection in the California Coast Range climate.

Activation of a Maturity Gene

Sorghum vulgare is a 10-chromosome short-day tropical crop plant that is native at low latitudes where days are short the year round. Milo is a grain type of this species that has been cultivated in the United States for the past hundred years or more. During that time agricultural selection has reduced milo to a short plant that can mature as far north as the 45th latitude. The details of what occurred in the way of loss of biotypes during the early years of this selective period are unknown, but Quinby and Karper (1945) investigated the genetic differences between several maturity types developed during the last 50 years.

Dwarf white milo, a medium early strain, differs from Sooner milo, an even earlier one, by possessing a maturity gene M_1, which is absent in Sooner, m_1. The M_1 gene is inactive at a 10-hour photo-period, where the two varieties come into flower at the same time and develop the same number of leaves below the inflorescence. In contrast, at a 14-hour photo-period, the Sooner variety remains essentially unchanged, but the Dwarf white delays flowering about 22 days and produces about 11 more leaves below the inflorescence. The M_1 gene for the late flowering is dominant, and the F_1 hybrid between the two varieties behaves like the Dwarf white, modifying at the long-day condition.

If the F_2 plants develop under a 10-hour photo-period there is no segregation, and all plants correspond to the Sooner parent. Distinct segregation occurs, however, at the 14-hour photo-period in the rate of 3 late, tall : 1 early, short, as shown in Table III. The delaying dominant gene, M_1, is therefore latent at the short day and would have no effect at the native low latitudes of *Sorghum* although it has a very noticeable effect when the crop is moved to a higher latitude.

By crossing strains that had not been grown commercially for the last 50 years, Quinby and Karper discovered two more genes that belong in the system of non-linked genes that regulate maturity in *Sorghum*. As shown in Table IV, these genes, M_2 and M_3, strongly enhance the delaying effect of M_1, but they are not expressed unless M_1 is present and has been activated by the long-day effect.

The *Sorghum* example should be typical of many other plants outside their natural latitudes. A further delay in the milos occurs when they are moved to a cooler climate at a higher altitude. The maturity genes operate in a manner that would suggest action of chemicals that delay or inhibit floral initiation. The activation of the controlling gene, M_1, depends upon a certain photo-period, but the rate of the process should depend upon the temperature of the environment.

Latent Variability in Ryegrass

Wimmera ryegrass is a highly purified strain of *Lolium rigidum*, a 7-chromosome species native to the Mediterranean region. It is a spring annual that attains flowering without cold treatment. One would therefore not expect vernalization to have any effect on such a species.

J. P. Cooper (1954) found that when Wimmera ryegrass is sown outdoors in spring it will initiate flowering at the seventh to the eighth node (mean 7.2). When

TABLE III

MATURITY IN MILO: P_1 – SOONER, EARLY ($m_1 \ldots$) × DWARF WHITE, INTERMEDIATE ($M_1m_1m_3$) P_2*

Photo-period (hrs.)	No. of days from planting to flowering in F_2													
	42	45	49	53	57	61	64	67	71	75	79	83	87	Total
10	4	34 P_1 P_2	16	8	5	..	..	..	..	..	..	..	..	67
14	..	..	1	2	11 P_1	3	..	6	3	8	22 P_2	9	4	69

*Quinby and Karper, 1945.

TABLE IV

DAYS TO FLOWERING AT CHILLICOTE (14 hrs.)*

Phenotypes	51	66	82	96
Early, m_1	51			
Intermediate, $M_1m_2m_3$		66		
Late, $M_1m_2M_3$			82	
Ultra-late, M_1M_2				95–97

*Quinby and Karper, 1945.

it is sown in a heated greenhouse and given continuous light, considerable previously hidden variability is disclosed, and the seedlings initiate flowerng at various nodes, ranging from the fourth to the twenty-first, as shown in Table V (mean node, 12.5). The variability will again be hidden if, before the continuous light treatment, the seeds are vernalized for six weeks at 3° C. When the cold treatment is followed by continuous light, the plants will initiate flowering at the fourth to the sixth node (mean 5.1).

TABLE V

WIMMERA RYEGRASS, *Lolium rigidum*, LATENT VARIABILITY REVEALED AT CONTINUOUS ILLUMINATION*

	Node of floral initiation									
	4–5	6–7	8–9	10–11	12–13	14–15	16–17	18–19	20–21	Total
Vernalization at 3° C.	11	9	..	..	..	..	..	..	..	20
Heated greenhouse only	2	..	4	7	7	5	1	2	2	30

*After Cooper, 1954.

The cold treatment apparently starts and equalizes certain gene-controlled processes related to development. No previous selection has taken place for the combination of heated greenhouse and continuous light, and the plants accordingly reveal a considerable degree of residual variability under this artificial condition.

HEREDITY AND ENVIRONMENT REGULATING DATE OF FLOWERING IN *Potentilla*

Potentilla glandulosa is a 7-chromosome western North American perennial plant of the rose family which along the 38th latitude in California has evolved four morphologically distinct subspecies and at least eight ecotypes. The ecotypes are

fitted to altitudes from sea level to 3,500 meters and include winter-active forms in the lowlands and winter-dormant ones in the mountains. When transplanted to the same garden, they differ in the date at which they begin flowering.

A plant of the foothill ecotype from 800 meters altitude was crossed with another of the subalpine from 3,100 meters. The F_1 plants were selfed, and 578 plants of an F_2 were cloned and cultivated at three altitudes: Stanford near sea level, Mather at 1,400 meters, and Timberline at 3,000. The dates when first flowers appeared on each of the parental, F_1 and F_2 clones at each of the transplant stations were noted and averaged for four years. At any one station the relative earliness was approximately the same in successive years for each F_2 individual.

The frequency curves of the variation in the date of flowering at the three stations are graphed in Figure 1. At each station there was highly transgressive segregation. Both parental plants were among the earliest of their ecotypes, and at Stanford the subalpine parent was one week earlier than the foothill. The spread in time of flowering in the F_2 was seven weeks at Stanford with equal transgression towards earliness and lateness. At the mid-altitude station the same population had a similar transgressive spread, but the transgression was towards lateness.

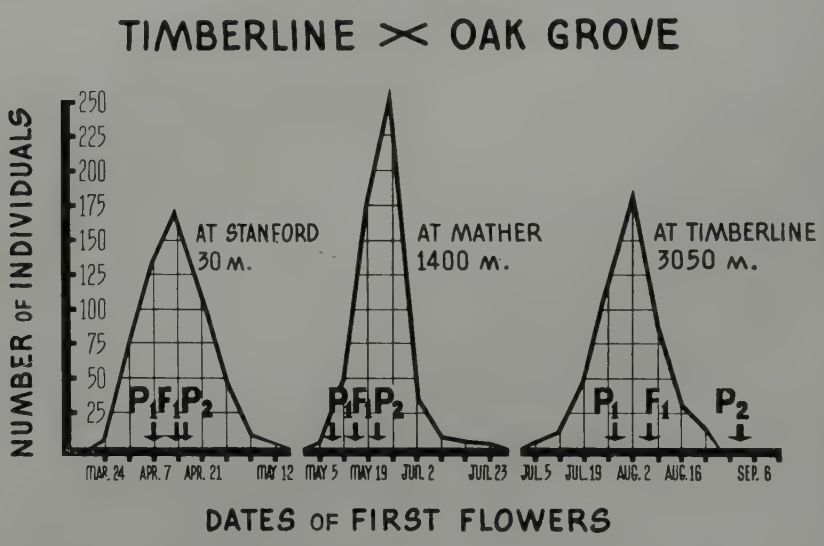


FIGURE 1. Frequency diagrams of mean date of first flowers among 511 F_2 progeny of subalpine × foothill ecotypes of *Potentilla glandulosa* cloned and grown at the Stanford, Mather, and Timberline stations. The segregations are transgressive, and the direction of the transgression shifts from station to station. (From Clausen and Hiesey, 1958.)

At the high altitude station the foothill parent flowered exceptionally late in the season based on observations in two years when it was able to survive one winter. In five other attempts at transplantation it died the first winter. At Timberline there was the usual spread among the F_2's, but all transgression was towards earliness. Many hybrids were therefore earlier than the early Timberline parent. A great many of the F_2's are markedly successful at the high altitude and have survived there for twenty years.

Among the F_2's there is practically no correlation between the earliness of a plant at one station and its earliness at the other two. This finding is obvious from the graphs in Figure 2. For example, the earliest group of F_2 plants at Stanford consists of 64 clones. These 64 separate at Mather into four groups ranging over an interval of nearly one month, and each of the Mather groups separates again at Timberline; the Mather May 25 group, for example, has at Timberline a spread from July 8 to August 19. All the other groups of earliness at Stanford separate in similar manner at the other two stations.

It is obvious that the processes that determine the dates of flowering are inherited, but the processes that determine them at Stanford are not the same as those that regulate them at Mather or at Timberline. Some of the station-to-station shifts must be due to shifts in the rates of gene-regulated processes in these highly contrasting environments, but it is highly probable that some of the genes do not express themselves at one or the other of the stations. The conditions at one or the other of the stations may be below or above the threshold at which a particular gene becomes activated.

Seed Dispersal in *Geum*

Two kinds of seed dispersal have evolved in *Geum* and in related genera of the rose family: by the fur and feathers of animals and by the wind. Subgenera within *Geum* are distinguished by these major dispersal methods, and the species within the subgenera are distinguished by variation in the details of the dispersal.

Geum fruits have very long styles that become hardened and persist. The subgenus *Eugeum* has animal dispersal. About midway on its style a loop develops, and an abscission layer is formed in the loop so that the tail half of the style becomes detached and the remainder persists as a hardened beak ending in a hook that attaches to the fur of animals. The subgenus *Sieversia* (here taken in Willdenow's original conservative sense) has a straight style ending in a feathery fruit tail that remains attached, providing the fruit with a sail that can drift with the wind.

Gajewski (1952, 1957) crossed the 21-chromosome *Geum rivale*, having a hooked fruit beak, with the 14-chromosome *Sieversia montana*, dispersing by a feathery straight fruit tail. The hybrid was partially fertile, had 14 pairs of chromosomes in addition to seven singles, and the F_2 population was large enough to provide some indication of the mode by which gene systems regulate the morphological traits that determine the seed dispersal method. The recombinations indicate that many genes contribute to the differences between the two kinds of fruit appendages: the styles of the F_2 progeny varied in their degrees of featheriness, straightness or curving, abscission or no abscission, in total length, and in the ratio between the persistent and detached parts of the style.

In the two parental species the genetic controls are very firm, but the control mechanism is less decisive in the hybrid. In the beginning of the flowering season, the F_1's produce fruits similar to those of the *Sieversia* parent, but later in the season the styles become curved, and their tails may or may not become detached. At this stage the fruits in the center of the flower have long, straight, non-detaching feathery tails similar to those of *Sieversia*, and the marginal fruits are similar to those of *Geum*. Some flowers have all three kinds of fruit together.

No two among the 96 F_2 progeny were alike in regard to their fruits. The majority, 52 plants, had fruits with hooks and detached tails, resembling somewhat those of *Geum*; 20 had fruits of this and the intermediate kind interspersed in the

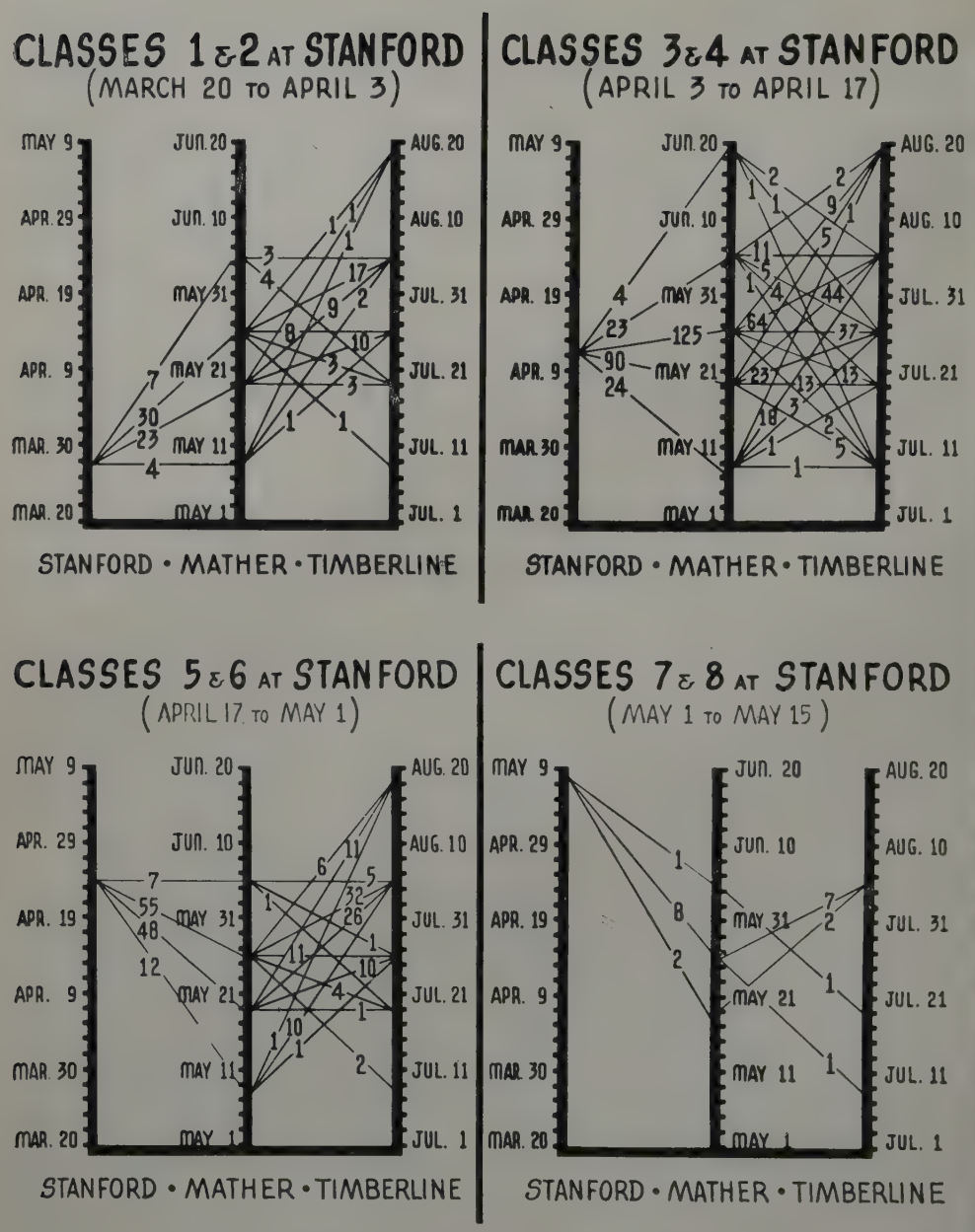


FIGURE 2. Graphs showing recombinations and frequencies in patterns of earliness in F_2 clones of subalpine × foothill ecotypes of *Potentilla glandulosa* at Stanford, Mather, and Timberline. The numbers on the lines indicate the frequencies in each category. *Upper left*: the fraction that is early at Stanford; *upper right*: mid-early fraction; *lower left*: a late-flowering fraction; *lower right*: the latest flowering fraction at Stanford. (From Clausen and Hiesey, 1958.)

same flowers; six had a spectrum of all three kinds of fruit in the flower, similar to the F_1; seven F_2 plants had fruits of the straight feathery kind interspersed with intermediates; finally, the fruits of eleven plants were exclusively of the *Sieversia* kind and had straight, persistent, feathery tails. It was obvious that the fixed patterns represented by the parental species had been broken up through the gene interchange.

A fair number of genes appear to regulate the development of the styles in the parental species. The regulation operates through growth processes, and the penetrance changes with the developmental stage of the plant and with the position of the fruit on the receptacle, suggesting transport and diffusion of chemicals.

The genetic results indicate that the two ecologically important methods of seed dispersal have evolved gradually through selection between small steps. The two kinds of fruit may have had their origin in the intermediate kind that is labile enough so that selection can take place in opposite directions. Such selections could finally anchor the two balances as we know them today.

Conclusion

Diversified populations, ecotypes, subspecies, and species carry great potentials for evolutionary variability through their systems of genes. The systems are rich in complementary, inhibitory, and other genes that are not expressed phenotypically. The genes are arranged in hierarchies of epistatic and hypostatic action, and the intensities of their phenotypic expressions vary with the environment. Certain genes even have definite thresholds for their activation. The widespread occurrence of such gene systems indicates that the sum total of evolutionary potentials through recombinations is far greater than commonly estimated.

The evolutionary potentials are being released as changes in the environments over geologic periods cause ecotypes to migrate to new areas and to become cohabitant with others that previously were geographically remote. Changes in environment provide the moving forces in evolution. Migrations may result in activations of hitherto inactive genes, followed by genic recombination, by natural selection and by adjustment to the new environment through evolution of a different composition of biotypes.

References

Clausen, Jens. 1951. Stages in the Evolution of Plant Species. Ithaca, N.Y.: Cornell University Press. Pp. 206.

Clausen, Jens, and William M. Hiesey. 1958. Experimental studies on the nature of species. IV. Genetic structure of ecological races. Carnegie Inst. Washington, Pub. 615, 312 pp. (contains detailed references to the literature on the subject).

Cooper, J. P. 1954. Studies on growth and development in *Lolium*. IV. Genetic control of heading responses in local populations. J. Ecology *42*: 521–556.

Gajewski, W. 1952. The hybrids between two subgenera of *Geum* L. Acta Soc. Bot. Poloniae *21*: 489–516.

——— 1957. A cytogenetic study of the genus *Geum* L. Soc. Bot. Poloniae, Monographiae Botanicae, IV, pp. 416.

Quinby, J. R., and R. E. Karper. 1945. The inheritance of three genes that influence time of floral initiation and maturity date in milo. J. Am. Soc. Agron. *37*: 916–936.

THE CYTOGENETIC BASIS OF SPECIATION IN COLEOPTERA[1]

Stanley G. Smith[2]

THE NEARLY seven hundred species of beetles now known cytologically, in embracing some three hundred genera in thirty-eight families, provide a revealing cross-section of the genetic systems and chromosomal variability present in the order as a whole. The most widespread type is a bisexual species having nine pairs of autosomes and an XX (♀) : XY (♂) sex-determining mechanism. In it, the metacentric Y is extremely minute and associates terminally with the relatively large X to form a ring bivalent, which in side view at metaphase has the appearance of a parachute (Xy_p). If this 9 AA + Xy_p was the ultimate condition in protocoleopterous forms of the Triassic, extensive structural rearrangements must have occurred to account for the currently known diploid limits of 12 and 60.

Although the evolutionary history of the more remotely related cytological extremes can never be established with certainty, some light must surely be shed on how these have come into being by a study of the principles operating in present-day species, for most assuredly these are in no way different from those that have prevailed in the past. A first avenue of approach to an elucidation of this problem of caryotype evolution is obviously through a comparison of groups of closely related species that cover a wide and continuous range of chromosome numbers especially if, as for example in weevils of the genus *Pissodes*, some of the species exhibit chromosomal polymorphism. Parenthetically, it should be noted that when differences in chromosome number are extreme but differences in external morphology are slight, as even between certain subspecies, the apparent absence of forms with intermediate numbers may well be no more than a reflection of inadequate sampling. Thus the coccinellid *Mulsantina picta picta* (Rand.) of Ontario and Quebec was originally recorded, in 1953, as having $2n = 12$ chromosomes; in California *M. p. minor* (Csy.) was recently found to have $2n = 20$ chromosomes, including an Xy_p sex-chromosome pair—there it is cytologically a typical coccinellid. Extending the area of sampling this year, a neo-XY *Mulsantina* was discovered in the interior of British Columbia and in the Yukon with $2n = 18$ chromosomes—it is confidently expected that eventually forms with 16 and 14 chromosomes will be encountered.

Conclusions based on simple morphological comparison of caryotypes, although often almost certainly correct, require in the long run the reinforcement possible from a study of the pairing relations in hybrids between the species concerned. As a general rule, however, where hybridization of beetles has been successful, the chromosomes are too small to lend themselves to detailed analysis. Fortunately this is far from true in the North American species of ladybird beetles in the genus *Chilocorus*—their chromosomes are large, about ten microns between the centromeres of metaphase bivalents, and are fully endowed with character. I, therefore,

[1]Contribution No. 494 from the Division of Forest Biology, Science Service, Department of Agriculture, Ottawa, Canada.

[2]Head, Section of Cytology and Genetics, Forest Insect Laboratory, Sault Ste. Marie, Ontario, Canada.

propose to discuss some cytogenetic investigations into the evolution of this complex of species which have been carried out over the past year while I was a guest of the Department of Biological Control, University of California, and which are still underway at Sault Ste. Marie. The general conclusions I have reached, based on almost 800 crosses and 1,500 inadequately studied slides, may require amendment after the cytological picture has been fully elucidated, but it is believed that they are substantially correct.

The type species, *Chilocorus cacti* (L.), is restricted in distribution in North America to the extreme south of the United States. A second species, *C. stigma* Say, at first thought to occupy the whole of North America, was considered by Leconte, in 1857, to be replaced in California by the closely similar *C. fraternus*. Some forty years later, Casey recognized another closely similar species in California and named it *C. orbus*. The two California species overlap marginally, the former being found in the central valley, the latter mainly along the coast. Although Leng, the outstanding authority on North American Coleoptera, refused to acknowledge *fraternus* and *orbus* as "good" species, recent unpublished taxonomic studies by John Drea at the University of California have strongly confirmed their validity. Moreover *C. stigma*, it now appears, is also absent from British Columbia, and thus appears to be restricted to the east of the Rocky Mountain Range or, in the United States, to the east of the Sierra Nevada Mountains.

C. stigma comprises an assemblage of remarkably different forms that exhibit a high degree of chromosomal polymorphism. This polymorphism finds its basis in both morphological and numerical variation, differences that are clearly causally related. Fundamentally the diploid number is 26. Decrease in number is brought about by centric fusion of non-homologous chromosomes; increase by the presence of supernumerary chromosomes or chromosome arms floating in the species in variable numbers. Changes in shape arise from the complete loss of, or partial loss from, one arm of the autosomes, or at meiotic metaphase by the substitution of a ring- or V-shaped configuration for two rod-shaped bivalents.

All the variables contributing to the total polymorphism are rendered physiologically and genetically acceptable to the species through the restriction of the genetically active euchromatin to one of the two equal-sized arms of each unfused chromosome, the other being totally heterochromatic. This interpretation is based on the dispensability of the one arm, its failure to form chiasmata, its differential staining, and its differential reaction to low temperature. Now, dislocation of the two arms through the centromere of two such chromosomes (Ae.Ah and Be.Bh) can give rise to various telocentric and, after rejoining, metacentric derivatives. Among these Ae.Be will be a new, wholly euchromatic metacentric chromosome; Ae. and Be. will be the wholly euchromatic telocentrics that appear in heteromorphic bivalents; and Ah., Bh., and Ah.Bh, the telocentric and metacentric by-products of fusion that either are eliminated or float in the population as supernumerary chromosomes. At meiosis chiasmata are formed only between euchromatic arms: consequently, supernumerary chromosomes fail to pair and segregate at random; unfused bivalents are rod-shaped, homozygous fused bivalents are ring-shaped, and the trivalent in the fusion heterozygote is V-shaped. Thus, with full pairing the chiasma frequency per cell is invariably 13, irrespective of the degree of polymorphism.

It is of interest to note that this highly unusual type of chromosome is found, so far as is known, throughout the whole of the tribe Chilocorini, and also that the differences in chromosome number that characterize species of such genera as

Exochomus have likewise been acquired by an identical process of fusion. It is also interesting that the dual nature of the unfused chromosomes allows contravention of Robertson's "law" of numerical constancy of chromosome arms, the number (Matthey's "N. F.") being reduced from fifty-two arms by four as each fusion becomes homozygous. Theoretically, with a maximum of six fusions possible, this Robertsonian imbalance should reach an ultimate low of twenty-eight arms—a condition that, it will be seen, has in fact already been realized in North America.

If the floating supernumeraries and the heteromorphic bivalents are disregarded, *stigma* is monomorphic in Florida: the females have 26 chromosomes, the males 25. This numerical difference results from the sexes being determined by an $X_1X_1X_2X_2$ (♀) : X_1X_2Y (♂) sex-chromosome system, which itself arose from the Y-chromosome having fused with an autosome, the homologue of which, segregating with the X_1, became in essence a second sex chromosome, X_2. North of Florida, the first purely autosomal fusion makes its appearance in populations in the State of Maine; it had earlier been recorded from Kentville, Nova Scotia, and was accordingly named the Kentville fusion. It has been found to date only in the heterozygous condition in these two eastern regions and involves about 8 per cent of the pertinent chromosomes. In Ontario, a second, morphologically distinct autosomal fusion occurs at Vineland, near Niagara Falls: it was present only in the heterozygous state in 6 per cent of the population, so that this, the Vineland fusion, had a frequency of 3 per cent, and the accompanying Kentville fusion frequency had risen to 14. Farther west in Ontario, in a small local population near the Agawa River, some 100 miles north of Sault Ste. Marie, the Kentville and Vineland percentages were 27 and 36, respectively. At Morden, Manitoba, about 70 miles southwest of Winnipeg, a third autosomal fusion, again morphologically distinct, is found together with the more easterly pair—their percentages were approximately Kentville 68, Vineland 23, and Morden 20. Finally at Conquest, Saskatchewan, the most westerly point sampled, a small, restricted population of *stigma* proved to be homozygous for the Kentville fusion, the Vineland fusion had increased to 29 per cent, and the Morden one to 44 per cent. It is thus evident that *C. stigma* Say is a complex of cytologically different forms that, in progressing north and west from Florida, ranges from a simple monomorphic entity ($2n\,♂ = 25$) to a highly polymorphic one in Manitoba and Saskatchewan in which males may possess any number of chromosomes from 25 down to 19 and any one of 27 ($= 3^3$) different caryotypes. Moreover, the frequencies of the three fusions are seen to increase steadily in going west from the eastern seaboard. The species, therefore, shows what I choose to call sequential chromosomal polymorphism and aptly illustrates the maxim "Chromosal diversification facilitates territorial invasion."

All the other North American *Chilocorus* species, in common with those of the remaining genera in the Chilocorini examined cytologically, have a neo-XY sex-determining mechanism derived from the Xy_p system found almost universally in the Coccinellidae. They are also all homozygous for at least two fusions: *cacti*, *fraternus*, and *orbus* each have two, *tricyclus* has three, and *hexacyclus* has six, the maximum number possible. The last two are recently discovered species that occupy the interior of British Columbia and, surprisingly, the eastern foothills of the Rocky Mountains in Alberta respectively; although it is possible that *hexacyclus* was transported across the mountains by human agency. In *orbus*, *tricyclus*, and *hexacyclus* the heterochromatic arms are essentially uniform in size and shape throughout the three complements, except that, whereas *orbus* and *tricyclus* have apparently telocentric neo-Y chromosomes, that of *hexacyclus* is sub-metacentric. In *cacti* and

fraternus, on the other hand, the heterochromatic arms vary morphologically, the two species being indistinguishable in this respect. In these five species, which collectively may be termed the "cyclus" complex, three different diploid numbers are therefore encountered: 22, 20, and 14. Connecting forms with 18 and 16 chromosomes doubtless existed some time before *hexacyclus* originated; possibly they still do, for our knowledge of the cyclus group is still meagre and the Great Basin, between the Sierra Nevada Mountains and the Rocky Mountains, is from this point of view entirely unexplored.

Under laboratory conditions, hybrids between *orbus, tricyclus,* and *hexacyclus* are easily obtained and their cytology reveals, by the pairing relations between chromosome arms at first meiotic metaphase, that their fusions are truly homologous from species to species. Thus the three have a common line of descent—they are monophyletic just as the *stigma* complex is. However, although pairing in these hybrids is essentially perfect, the three chromosomes involved in trivalent formation encounter such difficulty in orientating correctly that high degrees of sterility, even exceeding 50 per cent, are inevitable.

Although under experimental conditions *cacti* and *fraternus* will mate readily with *orbus*, *tricyclus*, and *hexacyclus*, the resulting eggs are without exception sterile. Matings between *cacti* and *fraternus* produce viable progeny only if the smaller *fraternus* is used as the male. The reciprocal mating always results in the premature death of the female, presumably because of profound size differences between their genitalic structures. Meiosis in hybrids from *cacti* females crossed with *fraternus* males is, however, so regular that it is entirely indistinguishable from that in the pure species, and thus establishes not only the complete homology of the two fusions but also that of the unfused chromosomes in their respective complements. Little is as yet known regarding the fertility of the F_1 progeny, other than that both inbreeding and backcrossing to *fraternus* males result in some, at least, of the eggs being viable.

In the laboratory *stigma* will also cross freely with all five members of the cyclus group, but progeny are realized only in combination with *orbus*, *tricyclus*, and *hexacyclus*. Analysis of the pairing relations at first metaphase of spermatogenesis in these hybrids, the only time at which the composition of the various configurations can be satisfactorily determined, shows, first, that one of the two *orbus* fusions, o_1, is homologous with the Kentville fusion in *stigma*: we may therefore say that both o_1 and the Kentville fusion consist of chromosome "1" plus chromosome "2." The second *orbus* fusion, o_2, instead of forming a ring with the Vineland fused chromosome, however, forms a chain of four chromosomes, thus showing that o_2 involves chromosomes "3" and "4" and the Vineland fusion chromosomes "4" and "5"—the two are only semi-homologous. Although some uncertainty exists at the moment concerning the homologies in the Morden fusion, there can be no doubt that it is entirely unrelated to the third, *tricyclus* or t_3, fusion. When *tricyclus* female is crossed with monomorphic *stigma* male, the resulting males carry a chain-of-four besides the two trivalents formed with o_1 and o_2. Since a minimum of six euchromatic arms are essential for the formation of the three chiasmata required in a chain of four chromosomes, then two of the four components of the chain must be fusion chromosomes derived from opposite parents: it, therefore, follows that t_3 must be semi-homologous with the Y of *stigma,* the only fusion chromosome carried by it when monomorphic. Consequently, if we label the Y of *stigma* "12" plus neo-Y, t_3 must be composed of chromosomes "11" plus "12." It is, moreover, evident that it was chromosome "12" in the progenitor of *stigma*

that initiated the fusion process in the east by uniting with the original neo-Y to form the present-day X_1X_2Y sex-determining mechanism. It will be appreciated that these particular homologies can be demonstrated only when *stigma* is used as the male parent: the hybrid male thereby acquires a new, synthetic complex, $X_1X_2Y_1Y_2$, in which the fourth entity, Y_2, is chromosome "11" of *stigma*. By the reciprocal combination only a neo-XY system can be perpetuated.

The Kentville and Vineland fusions in *stigma* and the three fusions in *tricyclus* being now accounted for, it is necessary to turn to hybrids involving *hexacyclus* in order to determine the constitution of the Morden fusion. Persistent difficulties have been encountered in efforts to do this because cultures homozygous for the Morden fusion have not as yet been selected out of the Morden stock and, as will be shown later, the Conquest stock proved to be genetically contaminated. Nevertheless, it seems that the Morden fusion is probably homologous through both arms with one of the *hexacyclus* ones, rather than semi-homologous, as was first thought to be the case.

In all crosses between *stigma* and the various cyclus species, the resulting hybrids, as expected, are highly sterile. The sterility is doubtless attributable to a number of different causes, the most obvious cytological components of which are, first, failure of pairing, presumably in part because of segmental differences between homologous, or homeologous, chromosomes, and, second, irregular disjunction following mal-orientation of trivalents. However, much of the failure of pairing seemed to be physiologically conditioned, for the over-all chiasma frequency is noticeably increased following storage of adults at sub-normal temperatures. Pending intensive study of the cytology of these hybrids, however, no serious attempt will be made to classify the various abnormalities encountered, or to use their chiasma frequencies as a yardstick in the assessment of relationship. Suffice it to state, first, that, although considerable numbers of first and second division bridges were observed, none of these was accompanied by a fragment and thus they are presumably not the result of chiasmata being formed between relatively inverted segments. Second, there is evidence of at least one fairly extensive duplication but, because more than one chiasma is never formed in any one arm, other smaller translocations could exist without betraying their presence.

The complete sterility resulting from crosses between *cacti* or *fraternus* and the other members of the cyclus complex demonstrates that a reproductive isolation barrier has been evolved to maintain the integrity of the partly sympatric *orbus* and *fraternus*. Whatever the nature of the barrier, the high viability and the regular meiosis of *cacti* $\times$ *fraternus* hybrids point to *orbus* as the "carrier" species. Furthermore, it is obvious that it functions also, and quite superfluously, between *fraternus* (and *cacti*) and the geographically isolated pair of species, *tricyclus* and *hexacyclus*; it functions not to prohibit gene exchange between them in nature, but because the latter pair have inherited the barrier from their *orbus* forebears—it is a relic of their past ancestry.

Following up this trend of thought, it is tempting to speculate that *stigma*, in developing a genetic system that prevents successful hybridization with eastern populations of *cacti*, has in fact evolved one that is precisely the same as that acquired by *orbus* to prevent gene exchange with *fraternus*. In a word it would seem that, where not protected by sound geographical or effective ecological barriers, the integrities of the various North American species of *Chilocorus* are maintained through the operation of absolute reproductive isolation barriers.

Moreover, once evolved, a barrier becomes an integral component of the genotype and is handed down to descendent species.

If the cytological and external morphological evidence that *hexacyclus* is in reality a western species is correct, it becomes of particular interest in the light of the foregoing to examine the results of an actual breakdown in an otherwise inviolable geographic isolation barrier. Briefly, the case history is as follows. In the autumn of 1956, eleven adult males in a collection received from a schoolyard at Conquest, Saskatchewan (the population already referred to), all proved to be typical polymorphic *stigma*, except that all were homozygous for the Kentville fusion. The following autumn, a further collection from the same small population included a number of adult males that showed degrees of cytological heterogeneity far too extreme to be classified as *stigma*-type polymorphism and that, in the light of later observations, were undoubtedly hybrids between the original schoolyard population and *hexacyclus*, the existence of which at that time was entirely unknown. Shortly thereafter a second shipment arrived from Conquest, this time not from the schoolyard but from a farm about a mile away. Upon examination, they all proved to be *hexacyclus*, indistinguishable cytologically from those obtained later from the foothills of the Rocky Mountains in Alberta. A dozen females were taken at random from each of the schoolyard and farm collections and set up individually for rearing of progeny. Those from the schoolyard produced (1) typical polymorphic *stigma* progeny, (2) hybrid progeny, or (3) progeny of both types. Those from the farm gave pure *hexacyclus*. It would, therefore, appear that *hexacyclus* had only recently invaded the schoolyard trees, possibly during the autumn of 1956, after the original collection had been made; they had then hybridized with the resident *stigma*, so that their progeny appeared among the sample received in the autumn of 1957. The schoolyard population, on the other hand, had failed to migrate to the farm. Thus the progeny of the schoolyard adults, having mated there or after arrival at the laboratory, could comprise pure *stigma*, or the F_1 or F_2 of *stigma* $\times$ *hexacyclus*, or backcrosses. It is, I suppose, possible that the *hexacyclus* was accidentally introduced into the Conquest area along with imported trees. However this may be, the schoolyard population in 1957 constitutes unequivocal proof that in nature, as in the laboratory, there is no sexual isolation between the two species: their normal geographical isolation has rendered the development of a sexual isolation barrier unnecessary.

This demonstration that introgressive hybridization is possible between species of *Chilocorus* opens up the question of whether, instead of being confronted with two distinct series that arose independently in the east and in the west by parallel processes of fusion, we are in fact concerned with the consequences of hybridization between two distinct species, a *hexacyclus*-type progenitor and a fusionless, neo-XY antecedent of *stigma*. Cytological evidence in favor of introgression is to be found only in their common possession of fusion o_1 and perhaps one of the *hexacyclus* fusions. Against it is the semi-homology of the o_2 and the Vineland fusions and that of the t_3 and XXY fusions, which embrace, of course, their different sex-chromosome mechanisms. Genetic evidence against a hybrid origin of the *stigma* complex is perhaps to be found in the possession by the entire cyclus complex of a dominant gene (or genes) determining larval scolal colour and its complete absence from *stigma*. Moreover, that the progressive incorporation of fusions in the cyclus group is an accomplished fact, a process duplicating that which long ago occurred in the differentiation of species of *Exochomus* and other

genera in the Chilocorini, argues in favour of the independent origin of the two series.

The question of introgressive hybridization being effectively weakened, we are now in a position to trace the steps through which the genetic systems and chromosomal changes observable in the North American species of *Chilocorus* have most probably evolved. As a starting point it would appear reasonable to assume, on the basis of world-wide distribution data, that this continent was invaded via the northwest some time before the last glacial period by a species similar to the Palaearctic *C. bipustulatus*, a species that is cytologically indistinguishable from *cacti* and *fraternus*, or by one like *C. renipustulatus*, adults of which resemble the North American species but whose cytology is unknown. With the onset of the Ice Age this invading species retreated to the south, ending up in Central America via the west and in Florida via the east. Following the return of temperate conditions *cacti* spread across the south of the United States, where it is now established in southern California, Texas, and the tip of Florida. Farther north *fraternus* occupied the central valley in California and *orbus* separated off along the seaboard side of the Coastal Range; *orbus* in turn gave rise to *tricyclus*, from which *hexacyclus* arose via tetracyclic and pentacyclic forms.

In the course of its colonization of the southeast, the two fusions in the progenitor must have dissociated in the production of a *stigma*-like form having 24 autosomes and a neo-XY sex complex. From this the true *C. stigma* Say was evolved by the formation of the "12" plus Y fusion, semi-homologous with the t_3 fusion of *tricyclus*. With northward progression one of the original fusions was re-formed as the Kentville fusion, to be followed by the Vineland fusion, semi-homologous with the second *orbus* fusion, o_2. Finally the Morden fusion appeared, semi-homologous or fully homologous with one of the *hexacyclus* fusions. It is difficult to escape the conclusion that this re-entry into more northern territories has been facilitated by the adaptiveness of the genetic changes inherent in the various fusions. In fact, that selective advantages are indeed conferred—that chromosomal polymorphism is a causative agent in evolution—is proved by the parallel and independent seriation of fusions in the west, where similar entities, arisen by the same process, are today established as recognized, or cytologically recognizable, species.

In *Chilocorus stigma* it is therefore possible to see evolutionary changes actually taking place in nature on a grand scale in a present-day, living species, the end results of which have already been achieved by the western complex of species. The chromosomes thus provide us with an indelible picture of the sort that the theory of evolution envisages but which the taxonomist has been consistently unable to provide.

SPECIAL LECTURES

1. ARNE MÜNTZING. A New Category of Chromosomes. Third Huskins Memorial Lecture
2. THEODOSIUS DOBZHANSKY. Genetics and the Destiny of Man
3. SEWALL WRIGHT. Genetics, the Gene, and the Hierarchy of Biological Sciences. Presidential Address

A NEW CATEGORY OF CHROMOSOMES*

Arne Müntzing[1]

THE DIFFERENT CHROMOSOMES, constituting a chromosome set, are usually not only qualitatively different, but also indispensable for the life of the organism. Changes in the number of such chromosomes strongly influence vigour as well as fertility, and in diploid species not only nullosomics, lacking one entire chromosome pair, but also monosomics lacking one member of a pair are as a rule non-viable. Trisomics, on the contrary, having one chromosome too many, are viable and less influenced by the presence of the extra chromosome. However, if one more chromosome of the same kind is added, the resulting tetrasomics are generally very poor in vigour as well as in fertility.

It is well known that lethality is not caused by the absence of an entire chromosome only. As a rule absence of a small segment or even a single locus is sufficient to cause the death of an individual, which happens to be homozygous for such a deficiency. Consequently, a chromosome set usually represents a delicately balanced unit of co-operating chromosomes, each segment of which must be present in order to secure normal viability.

It has long been known, however, that there is a difference between the genetically active and finely differentiated euchromatic segments of the chromosomes and heterochromatic segments, which stain differently and which seem to be less active or even quite inert. The genetic inertness or partial inertness of heterochromatin is especially well known with regard to the Y-chromosome in *Drosophila* and the corresponding sex chromosome in some other organisms.

The genetic inertness of the heterochromatin, especially in the sex chromosomes, forms a connecting link between the normal, regular, and active chromosomes and another type chromosome which I prefer to call *accessory*. This kind of chromosome is also known under the somewhat inadequate name of supernumerary; B-chromosome is another useful designation to distinguish these chromosomes from ordinary chromosomes or A-chromosomes. The accessory chromosomes have also been referred to as second-class chromosomes or even as ghost chromosomes. As we shall see, an exact definition which sharply distinguishes accessory chromosomes from ordinary chromosomes is difficult to formulate. However, we may say that *accessory chromosomes occur in variable numbers in some members of a population and may be absent in other individuals without noticeable effects. They are usually small and heterochromatic and do not pair with the A-chromosomes at meiosis*. It may also be said that in several plant species there are various mechanisms which tend to increase the number of accessories in each generation. The most widespread of these mechanisms is non-disjunction of the accessories occurring in the first division of the pollen grain.

*Third Huskins Memorial Lecture. This Lecture is sponsored annually by the Genetics Society of Canada and was given in 1958 as a special Congress lecture.

[1]Institute of Genetics, Lund, Sweden.

Occurrence and Ecological Importance of Accessory Chromosomes

Fifty years ago the occurrence of accessory chromosomes in an animal was first observed by Lutz (cf. Stevens, 1908) in the insect genus *Diabrotica*. The first observation of this phenomenon among plants was made in *Zea mays* by Longley (1927) and Randolph (1928, 1941), who made the distinction between the ordinary 20 chromosomes occurring in these plants and the supernumerary chromosomes, occurring in some individuals and in some populations or varieties with varying frequencies. Randolph denoted the ordinary chromosomes as A-chromosomes and the smaller supernumerary chromosomes as B-chromosomes. He also distinguished different classes of still smaller chromosomes derived from the B-chromosomes. According to their size they were called C-, D-, E-, and F-chromosomes.

Since the original findings were made in some insects and in maize, the occurrence of accessory chromosomes has been reported in an ever increasing number of species. Bosemark (1957b) recently examined eleven species of grasses and, despite

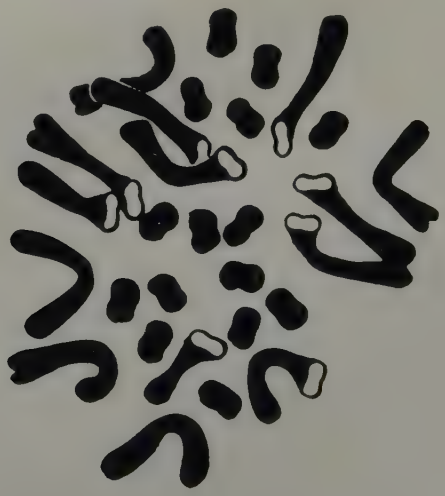


Figure 1. Somatic plate of a plant of *Festuca pratensis*, having 15 smaller accessory chromosomes of standard type in addition to the 14 normal chromosomes (from Bosemark).

limited sampling, found accessories in six of them (Fig. 1). Darlington (1955, 1956) states that hundreds of species are now known to possess accessory chromosomes and that we may surmise that a far larger proportion will later be found to have them. The literature in this field of research is already comprehensive and rapidly expanding. Under such circumstances I cannot cover the whole field in one lecture and shall therefore discuss only some recent findings of particular interest.

Especially thorough attempts to elucidate the ecological importance of accessory chromosomes have been made by my collaborators Bosemark and Frost. In *Phleum phleoides* the frequency of accessories was found to be markedly different in different populations (Bosemark 1956c), but it could not be determined whether this was due to ecological differentiation or only to historical circumstances. In *Festuca*

pratensis Bosemark (1956a) found a marked positive correlation between number of accessory chromosomes and clay contents of the soil.

A careful analysis of this material showed, however, that the apparently simple correlation between clay soil and number of accessory chromosomes is complicated and in some respects still obscure. The same is probably true of a similar correlation found by Fröst (1958a) in *Centaurea scabiosa* from a study of a large sample

FIGURE 2. *Left*: somatic plate of a plant of *Centaurea scabiosa*, showing presence of 13 small dot-like accessory chromosomes of standard type in addition to the other, larger chromosomes (from Fröst); *right*: first metaphase of meiosis (in side view) of a plant of *Centaurea scabiosa*, showing 10 unpaired accessory chromosomes scattered around the A-bivalents (from Fröst).

from Sweden and other parts of Europe. In Scandinavia and Finland there is a marked east-west difference in the frequencies of accessory chromosomes (Fig. 2). This geographical difference largely coincides with a difference in humidity, the frequency of accessory chromosomes being higher in arid regions. After careful consideration Fröst concludes that selective forces have probably played the greatest role in this differentiation, but historical factors and mere chance may also, to some extent, account for it. In this connection it is of interest that, in *Clarkia,* Lewis (1953) has found a similar correlation between adaptation to arid regions and presence of supernumerary chromosomes.

In rye, *Secale cereale*, my own work has revealed very considerable differences in the frequency of accessories in different populations. Such chromosomes (Figs. 3 and 4) are absent or rare in highly bred commercial varieties of western Europe but are more frequent in primitive strains from Asia. In Turkey, Afghanistan, and Iran accessory chromosomes are fairly frequent but may be absent in some populations. In north-eastern Asia they seem to be especially numerous (cf. Müntzing, 1957). In seven populations from Korea the percentage of plants with accessories was found to range from 19 to 91 (Figs. 7 and 8), and there is also other evidence indicating that in some strains in Korea accessories are present in almost every plant of the population. This may indicate that the accessories have a positive selective value in Korea in contrast to accessories of the highly bred European varieties, which, so far, have

FIGURE 3. Somatic plate of a plant of rye (*Secale cereale*), showing the 14 A-chromosomes (in outline) and 2 accessory chromosomes (black). The larger accessory chromosome is of the standard type, the smaller one has lost the terminal half of the long arm (from Müntzing).

FIGURE 4. A pair of standard accessory chromosomes of rye at pachytene, showing detailed differentiation into different regions (from Müntzing and Lima-de-Faria).

been found to have only deleterious effects on vigour and fertility (Müntzing, 1943). However, in plants raised at Lund from the original seed samples obtained from Korea the presence of accessories seems to be just as harmful as in Swedish rye varieties. Comparisons undertaken between plants without accessories and with different numbers of such chromosomes clearly showed that under our environmental conditions the plants without accessories are superior in vigour and fertility. These data strongly indicate that plants with three or four accessories are inferior to those having one or two.

Another fact of importance was gathered from a study of a summer rye strain from the Transbaikal area in Siberia. In the original seed sample obtained in 1951 the frequency of plants with accessory chromosomes was found to be as high as 28 per cent. During several years of cultivation at Lund the frequency of accessories

showed a slow but steady decrease and last year the percentage was only 18. This decrease is statistically significant. It will be interesting to see how far this spontaneous decrease in the number of B chromosomes will go, and at what level an equilibrium will be reached.

This problem has also been studied in another way. In 1953 a synthetic population of the same rye variety was constructed in which 10 per cent of the plants carried two additional accessory chromosomes. On account of the non-disjunction mechanism, which in rye occurs in the pollen as well as on the female side, an increase in the frequency of accessories in the offspring of the primary synthetic population was to be expected. This actually occurred, but the increase was much less marked than expected. Thus, the first daughter generation of the primary synthetic population showed an increase from 10 to 12.6 per cent of plants with accessory chromosomes and in 1957, three generations later, the value of 14.0 per cent was reached. This increase is statistically significant, but the very slow accumulation of plants with accessories in the synthetic population indicates that this increase is somehow counteracted by the environmental conditions. Probably the same ecological conditions which have reduced the frequency of accessories in the spontaneous population from 28 to 18 per cent have tended to counteract the increase in the synthetic population. It is probable that after some more years both populations will reach equilibria at the same level. It seems most likely that this level will be situated somewhere between 14 and 18 per cent, the values now characterizing the two populations.

Darlington (1955, 1956) was the first to point out that accessory chromosomes predominantly occur in diploid species, whereas such chromosomes are less frequent in polyploids. This correlation is assumed to be connected with the fact that diploid species are on the average more short-lived than polyploids. Darlington also believes that B-chromosomes may boost the variability and hence the adaptability of a species, such a boost being less useful in a polyploid. However this may be, the most recent literature on accessory chromosomes tends to support Darlington's observation that such chromosomes are more frequent in diploid than in polyploid species.

Genetic Effects

Since accessory chromosomes seem to be ecologically valuable in some populations, it would be desirable to demonstrate, in experimental material, some positive effects of such chromosomes. So far, however, such attempts have given remarkably meagre results and tend to show that in low numbers the accessories have very slight effects and in higher numbers they are always deleterious. However, these results vary from species to species.

For rye, the older data (Müntzing, 1943) as well as more recent results, almost without exception, demonstrate that the accessories are sub-inert but have harmful effects on fertility and vigor. This is noticeable even in plants with one or two accessories and is more pronounced when the plants have three or four such chromosomes. Above four or five the effects are very strong. Results of this kind were obtained not only in material from Swedish commercial varieties but also in material of primitive strains from eastern Asia. The negative effects of accessory chromosomes in material from Korea have already been mentioned, and similar results were also obtained with the strain from Transbaikal.

New and interesting material is represented by a strain of *autotetraploid rye*, carrying accessory chromosomes, which is now in culture at our institute. This strain

was produced by colchicine treatment of the diploid strain from Transbaikal. I expected that a higher number of accessory chromosomes would be tolerated in this autotetraploid strain than in the original diploid strain. However, the opposite result was obtained. Although measurements were undertaken only in euploid individuals with 28 A-chromosomes, even the addition of one or two accessory chormosomes was definitely harmful to seed setting, pollen fertility, plant height, and straw weight. Also, as recently observed by Sarvella (unpublished), the meiotic pairing of the accessories and their non-disjunction in the pollen mitosis are less well accomplished at the tetraploid level than in the corresponding diploid plants.

Energetic attempts have recently been made by Bosemark in *Festuca* and by Fröst in *Centaurea* to test the genetic effects of accessory chromosomes. As a rule the effects have been found to be negative, but Bosemark (1957a,b) showed that occasionally the presence of one or two B-chromosomes increased the vigor of the plants. This increase, however, seems to occur only in certain genotypes and under certain environmental conditions. Fröst (1958b) obtained similar results in *Centaurea*. Although the accessories in this species are small, and sometimes numerous, some indications but no definite evidence could be obtained that they are favourable in low numbers. In higher numbers they are clearly harmful.

Several other cases have been reported in which the accessory chromosomes are considered either to have little or no influence on the properties of the carrier or to have more or less deleterious effects. However, Rutishauser (1956a,b) has

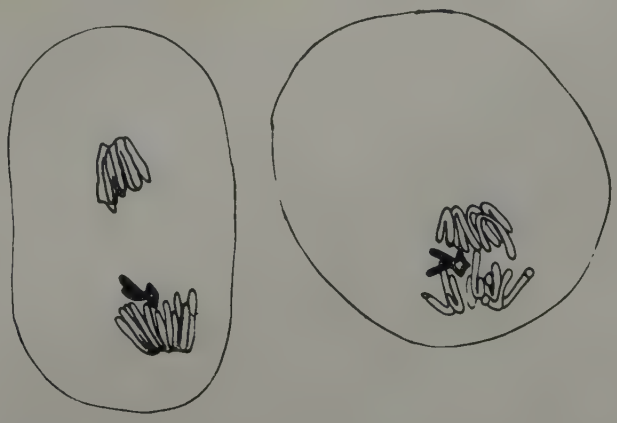


FIGURE 5. *Secale cereale* (left later stage than right) showing lagging and non-disjunction of accessory chromosomes at the first pollen mitosis.

reported that in *Trillium* seed fertility seems to be favourably influenced by the introduction of up to three euchromatic accessory chromosomes in the endosperm. He also found that seed setting in crosses between plants without accessory chromosomes was smaller than in crosses between plants containing such chromosomes. Though this observation probably needs to be repeated before it can be considered significant, it is supported by evidence from the genus *Achillea*, obtained by Ehrendorfer (1957). Artificial crosses between certain diploid species seem to succeed only when accessory chromosomes are present. This is in agreement with the fact that hybrid populations in nature, involving the same species, also contain accessory

chromosomes. Related polyploid species do not contain accessory chromosomes but are nevertheless able to hybridize with each other.

Mechanisms of Numerical Increase

Accessory chromosomes would certainly be rapidly eliminated from the populations, especially in material in which they have clearly deleterious effects, unless some other factor tended to preserve them. In rye this factor is the peculiar ability of the accessory chromosomes to undergo non-disjunction at the first mitosis in the pollen grain (Hasegawa, 1934; Müntzing, 1946) and also at the corresponding stage in the ovules (Håkansson, 1948). By this process (Fig. 5) both chromatids pass to the generative pole, and in the offspring the number of accessory chromosomes will therefore be about twice as high as expected (cf. Müntzing, 1949, 1954).

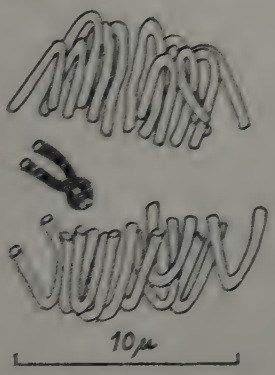


FIGURE 6. *Alopecurus pratensis* showing non-disjunction of accessory chromosomes at the first pollen mitosis (from Bosemark).

Rye is the only species so far known in which this type of non-disjunction is carried out in the pollen as well as in the ovules. However, Oestergren (1947) found the same mechanism in the pollen mitosis of a species of *Anthoxanthum*, and Bosemark (1954a, 1956c, 1957b) has revealed the occurrence of non-disjunction at the first pollen mitosis in six other grass species, belonging to the genera *Festuca*, *Briza*, *Holcus*, *Phleum*, and *Alopecurus* (Fig. 6). A similar mechanism, in *Poa timoleontis*, was recently described by Nygren (1957).

In *Zea mays* Roman (1947, 1948) found non-disjunction at the *second* mitosis of the pollen grain, which in combination with selective fertilization tends to increase the number of accessory chromosomes in this species. Non-disjunction has also been observed in *Sorghum purpureo-sericeum* by Darlington and Thomas (1941), but here it is limited to abnormal supernumerary divisions in the vegetative nucleus and does not lead to an increased number of B-chromosomes in the offspring.

Otherwise such a numerical increase is evidently characteristic of grass species with accessory chromosomes, though a few exceptions to this rule are known (*Poa alpina*, Müntzing 1948; *Poa trivialis*, Bosemark 1957b).

In rye various derivatives of the standard type of accessory chromosomes have been studied with regard to their ability to carry out non-disjunction. It has been

found that this is an all or none process. Some derivatives retain the non-disjunction ability completely while other derivatives are quite incapable of carrying it out. These different mechanisms are correlated with the presence or absence of a conspicuous knob near the end of the long arm of the standard accesssory. The presence of this knob seems to influence reproduction or separation ability at two other points on the chromosome (cf. Müntzing, 1954). Similar observations have recently been made by Longley (1956) with regard to B-type chromosomes in maize. In studies of translocations between the B-chromosome and the tenth chromosome Longley found that the presence of a major portion of the heterochromatin of a B-chromosome is necessary for the non-disjunction of a chromosome having the B-centromere combined with a segment of chromosome 10. Thus it seems reasonable to infer that the unusual behaviour of a B-chromosome in maize is related to the presence of a large modified block of heterochromatin.

The effect of heterochromatin blocks on the non-disjunction mechanism might be transmitted either along the same chromosome only, or else spread through the cytoplasm of the pollen grain, influencing other chromosomes as well. Bosemark (1956b) has demonstrated the validity of the latter alternative for his material of *Festuca pratensis*. Different derivatives of the standard accessory chromosome differ with regard to their ability to undergo non-disjunction in the first pollen mitosis. The important point is that these different derivatives influence each other when present in the same pollen grain. In this way a B-chromosome derivative which is incapable of non-disjunction when it is alone is forced to carry out this process when it is in the company of another B-chromosome type which is able to carry out the process when it is alone. Recently Dr. Håkansson informed me that he has seen the same phenomenon in rye. Thus, non-disjunction must be caused by some gene products diffusing into the cell and affecting certain regions of the various types of accessory chromosomes. This, however, occurs only in the special environments of the pollen grains and embryo sacs. All other mitoses are quite normal. Also in *Festuca* large heterochromatic segments are involved in the control of the non-disjunction process. They are also capable of inducing neo-centric activity in the chromosome ends.

Numerical increase by non-disjunction in the pollen mitosis is a phenomenon specific for the accessory chromosomes in grasses. In other groups, however, other mechanisms of numerical increase have been discovered recently. Thus, in *Trillium grandiflorum* Rutishauser (1956a) obtained experimental results which demonstrate that there is a mechanism of numerical increase on the female side. It is assumed that there is a directed movement of univalent accessory chromosomes at the meiosis in the EMC toward the dyad cell which forms the embryo sac and Rutishauser regards this as another mechanism for the maintenance of accessory chromosomes in wild populations.

Similar results were obtained by Kayano (1956a, 1956b, 1957) in *Lilium*. At first he found that a certain accessory chromosome was preferentially included in about 80 per cent of the egg cells and later on the cytological mechanism involved was directly observed. At the first division of meiosis the accessory chromosome in question is directed to the micropylar nucleus from which the egg nucleus ultimately results. In *Trillium grandiflorum*, on the contrary, the accessory chromosome is directed to the chalazal dyad cell which in this species gives rise to the embryo sac. Thus, as Kayano points out, it is obvious that the preferential segregation is closely correlated with the developmental polarity or gradient in the EMC. The situation is parallel with that in pollen grains, in which the first mitosis differen-

tiates the peripheral generative nucleus from the central vegetative nucleus. In this case the spindle is asymmetric and in species with non-disjunction of accessory chromosomes in the pollen this is assumed to be an important factor in directing the accessory chromosomes to the generative pole (Oestergren, 1947; Bosemark, 1954a, 1956).

Another example of numerical increase of accessory chromosomes has recently been discovered by Fröst (unpublished). In *Plantago serraria* chromosome counts in crosses revealed that there is a mechanism of numerical increase on the female side. Thus, among higher plants such mechanisms are now known not only in grasses and lilies, but also in the very different family *Plantaginaceae*.

In other groups less clear examples of numerical increase or numerical variation of accessory chromosomes have been reported. These involve mechanisms such as endomitotic duplication in the resting stage that precedes meiosis (*Polycelis,* Melander, 1950), or mitotic non-disjunction.

According to the previous survey different kinds of mechanisms of numerical increase are characteristic of accessory chromosomes. It should not be forgotten, however, that in some species such as *Centaurea scabiosa*, *Poa alpina*, and *Xanthisma texanum* such mechanisms are entirely lacking.

In *Centaurea* (Fröst, 1957) the rate of transmission is equal on the male and female sides and there is in general a small loss of accessories from one generation to the next. The loss of accessories, at least under certain conditions, must be compensated for by natural selection favouring plants with accessory chromosomes.

Somatic Elimination

Most species with accessory chromosomes show a regular behaviour of the accessories in somatic mitosis, and consequently the number of accessories present at meiosis is the same as the number present in the root tips. In some species such as *Zea mays* (Darlington and Upcott, 1941) and *Poa trivialis* (Bosemark, 1957b), there is a certain degree of somatic variation in number which has been thought to be due to weakness in the centromere of the accessories. Also in *Centaurea* Fröst (1956) finds that the standard accessory chromosome shows a slight variation in number within the plant. As there is no evidence in *Centaurea* that the centromere is weak, Fröst is inclined to believe that the somatic variation is caused by stickiness. This may sometimes prevent the separation of the daughter chromosomes and lead to numerical variation.

In sharp contrast to these plants with only a slight somatic variation in the number of accessories, a few plants are known in which the accessories occur only in certain tissues of the organism. Among higher plants this phenomenon was first observed in *Sorghum purpureo-sericeum* by Janaki-Ammal (1940) and Darlington and Thomas (1941). The second example was found by myself in a diploid strain of *Poa alpina* derived from Innsbruck in Austria (Müntzing, 1946, 1948). In the latter strain the accessory chromosomes are heterochromatic and smaller than the other chromosomes and are present in numbers ranging from two to eight in every individual of the strain. However, they are regularly eliminated from most of the somatic tissues and are retained only in the central parts of the plant (Fig. 7). Studies of root tips of germinating seeds (Håkansson, 1948; Milincovic, 1957) revealed that the accessories are generally present in the primary roots regardless of their length and stage of development, but they are completely

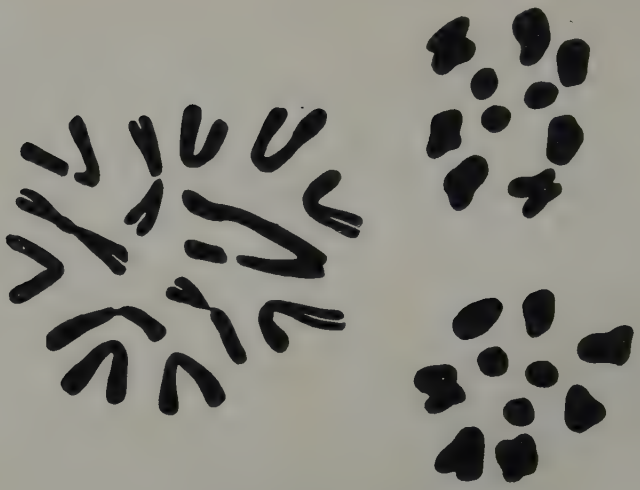


FIGURE 7. *Left*: somatic plate with 14 A-chromosomes of a plant of *Poa alpina*; *right*: first anaphase of meiosis in the same plant, showing presence of 6 smaller accessory chromosomes surrounded by the 14 A-chromosomes (2 corresponding plates with 7 A + 3 B in each). The accessory chromosomes are eliminated from most of the somatic cells (from Müntzing).

eliminated from all adventitious roots and are absent even in the young primordia of such roots. Consequently, they do not occur in root tips produced from cuttings and they are eliminated from the leaves.

A new sexual diploid strain of *Poa alpina* from Ardez in Switzerland has recently been studied by Müntzing and Nygren (1955). In this strain there are supernumerary chromosomes at meiosis which are not present in the root tips. Whereas the number of such chromosomes oscillates between two and eight in the Innsbruck strain previously mentioned, it is regularly two in the new strain from Ardez. These two chromosomes show regular pairing at meiosis and form an extra bivalent, which cannot be distinguished from the seven normal bivalents.

Some deviating plants in the Ardez strain were observed, the most interesting one having an eighth bivalent clearly smaller than the other bivalents. These additional small chromosomes were *not* eliminated from the root tips and apparently have a terminal centromere. They may be products of misdivision, and if so the factor or factors otherwise responsible for their somatic elimination must have been located in the segment or chromosome arm which has been lost. Thus, the larger original accessory chromosome which contains the elimination factor is present only at meiosis, while the smaller derivative is present in all tissues because it has lost the elimination factor.

Before leaving the genus *Poa* it should be mentioned that Nygren (1957) has recently observed somatic elimination of two accessory chromosomes in *Poa timoleontis*. The single plant so far examined had 14 chromosomes in the root tips and eight bivalents at meiosis.

A third example of somatic elimination of accessory chromosomes has recently been described by Berger and collaborators (Berger and Witkus, 1954; Witkus,

Lowery, and Berger, 1955; Berger, McMahon, and Witkus, 1955), in the composite *Xanthisma texanum* which has regularly eight chromosomes in the root tips. Of 100 plants examined only 35 had four bivalents at meiosis as expected, whereas 65 plants contained from one to four supernumerary chromosomes (Fig. 8). They occur in all cells of the embryo up to a certain stage, but then a differentiation sets in, and the accessories are eliminated by lagging in the cells of the root tissue.

FIGURE 8. Somatic chromosomes of *Xanthisma texanum*. V is an accessory chromosome with a terminal centromere, I–IV represent the normal chromosome complement (from Berger and collaborators).

Since the accessory chromosomes of *Xanthisma* have a perfectly normal mitotic behaviour in the shoot, it cannot be said that their centromeres are defective, but at the differentiation into root tissue some factor or condition makes it difficult or impossible for the accessories to react normally with the spindle.

ORIGIN AND NATURE OF ACCESSORY CHROMOSOMES

In animals there is evidence that accessory chromosomes are derivatives of sex chromosomes, and cases are known in which certain chromosomes are more or less intermediate between typical sex chromosomes and accessory chromosomes (cf. Melander, 1950; Virkii, 1954). As the sex chromosomes are often heterochromatic, more or less inert, and even with somewhat deviating and aberrant properties of the centromere, the difference between such chromosomes and supernumerary accessory chromosomes is not very great. By fragmentation and other structural rearrangements the newly derived chromosomes will lose the ability to pair at meiosis with the original chromosomes and as a rule they will become smaller.

Something similar seems to occur in different groups of moss species. Vaarama (1953a, 1953b) believes that the so-called m-chromosomes (minute chromosomes) are derivatives of sex chromosomes, and the m-chromosomes should then by new fragmentation give rise to the still smaller accessory chromosomes. In species of *Sphagnum*, Sorsa (1956) reports that the presence of many accessory chromosomes seems to disturb sex determination, and that the *Sphagnum* species in which the greatest numbers of accessories have been observed may sometimes be monoecious. This observation indicates that accessory chromosomes in mosses as in animals may be derived from sex chromosomes.

In animals, however, accessories may also be derived from autosomes. Virkii (1954) suggests that accessories may be formed as a by-product when species formation is associated with a change from a higher to a lower chromosome num-

ber. In such processes the genetically active, vital parts of a certain chromosome may be transferred by translocation to another chromosome of the set. The centromere and the proximal parts of the chromosome will remain and will continue to exist as a more or less inert accessory chromosome. Essentially the same hypothesis was earlier suggested by Avdulov and Titova (1933) and also in part by Darlington (1956b), who considers the accessory chromosomes to be derived recently or ultimately from the ordinary chromosomes. According to Darlington (1956b) the accessories may represent centric fragments or telocentric arms of A-chromosomes, having arisen by misdivision.

Several other workers believe that B-chromosomes arise by fragmentation of A-chromosomes. In *Oenothera* and *Caltha,* Cleland (1951) and Reese (1954) obtained data which support this hypothesis. In *Oenothera hookeri* some populations are characterized by the presence of two extra dimunitive chromosomes which tend to breed true. These chromosomes never pair with the other members of the chromosome complement, and Cleland suggests that they may have originated by a process of fragmentation, which has resulted in the loss of the pairing ends of a chromosome.

In *Caltha palustris* Reese actually observed certain small A-chromosomes undergo misdivision and give rise to two telocentric smaller chromosomes which look and behave like the ordinary B-chromosomes occuring in this species. It should be observed, however, that in this material it is evidently difficult to differentiate sharply between true B-chromosomes and real aneuploids containing supernumerary A-chromosomes.

In the genus *Clarkia* the difference between A- and B-chromosomes is also fairly arbitrary and Lewis (1951, 1953) has clearly shown that the B-chromosomes in *Clarkia* must represent duplications of part of the normal genome. Thus, a certain plant with 19 chromosomes instead of 18 was found to be a tertiary trisomic. The extra chromosome in this plant was cytologically comparable to naturally occurring supernumerary chromosomes and like them produced no detectable morphological difference. It should also be pointed out that the B-chromosomes in *Clarkia* are euchromatic and stain at mitosis and meiosis in the same way as the other chromosomes. This may be another indication of their recent origin. Darlington (1956b) suggests that the B-chromosome may become more and more heterochromatic in the course of evolution.

Resende and Da Franca (1946) have put forward the hypothesis that every chromosome in excess of the normal complement will become heterochromatic and hence genetically inactive. Accordingly, heterochromatin is considered as a dead weight on the nucleus affecting its metabolism as any parasite would do. Therefore, a change from an euchromatic to a heterochromatic condition would be a way by which the organism could neutralize the disturbing effects of supernumerary A-chromosomes. This would, of course, happen more easily in plants with vegetative propagation than in plants with normal sexual reproduction.

Fernandes (1949), working with *Narcissus bulbocodium*, has obtained some surprising but highly interesting results which indicate that an irreversible change from an euchromatic to a heterochromatic condition may occur all at once owing to the action of a single gene. Thus, according to Fernandes, a cross between a diploid individual carrying a dominant gene, causing heterochromatinization and a normal triploid without this gene gave F_1 plants in which the supernumerary chromosomes had become heterochromatic. This process inhibits the genetic action more or less completely and also decreases the activity of the centromere. Fernandes evidently

considers that different degrees of heterochromatinization may occur. If this process is total, the centromere will cease to function and the chromosome will be eliminated. If the process does not go quite so far, the centromere may function well enough to keep the chromosome going. It may then later acquire secondary adaptations such as a non-disjunction mechanism, which will tend to retain it in the population as a kind of parasite as originally suggested by Oestergren (1945).

This interesting theory is supported by cytological and experimental data. I do not understand, however, why the postulated gene affects only the supernumerary chromosomes and not all the chromosomes. Further data from *Narcissus* and similar results from other genera seem necessary before Fernandes' results can be generalized.

It is probable that accessory chromosomes have less marked genetic effects when they are heterochromatic than when they are euchromatic. This view is supported by observations obtained by Randolph (1941). Besides the B-chromosomes in maize Randolph studied, as we have seen, smaller derivatives of the B's denoted by other letters. One of these types, the D-chromosome, is much smaller than the B but at least as active. This is probably due to the fact that the D-chromosome is euchromatic, whereas the larger B-chromosome contains much heterochromatin, which may disturb or inhibit the action of the euchromatic segments in the same chromosome.

In the preceding account I have reviewed recent researches which may throw some light on the origin of accessory chromosomes. The differentiation between normal and accessory chromosomes has not been sharp, and consequently we may assume that accessory chromosomes in some species are of fairly recent origin. In other species, however, they must be quite old, perhaps as old as the other members of the complement. Such is the situation in several grass species and in rye, in which there is a definite standard accessory chromosome which has the same appearance in widely different populations of the world. Under conditions of cultivation this standard type frequently gives rise to various new derivatives through misdivision or deletion, but under natural conditions the standard type alone is successful. Bosemark (1957b), discussing this specialized, although extensively uniform organization, concludes that to achieve their possible positive functions, or even in order to be tolerated, the accessories must fulfil certain requirements which may be correlated with a special organization and morphology.

In spite of this special organization and morphology, the accessory chromosomes, as Darlington says, "break almost all the rules of genetics and physiology which rest on the uniform behaviour of A chromosome." On account of this unorthodox behaviour they represent interesting material for further work and certainly deserve our full attention.

REFERENCES

Avdulov, N. P. and N. Titova. 1933. Additional chromosomes in *Paspalum stoloniferum* Bosco. Bull. Appl. Bot., Genet., and Pl. Breed. Ser. II. *2*: 165–172.

Berger, C. A., and E. R. Witkus. 1954. The cytology of *Xanthisma texanum* D.C. I. Differences in the chromosome number of root and shoot. Bull. Torrey Bot. Club, *81* (6): 489–491.

Berger, C. A., R. M. McMahon, and E. R. Witkus. 1955. The cytology of *Xanthisma texanum* D.C. III. Differential somatic reduction. Bull. Torrey Bot. Club, *82* (5): 377–382.

Bosemark, N. O. 1954a. On accessory chromosomes in *Festuca pratensis* I. Cytological investigations. Hereditas, Lund. *40*: 346–376.

——— 1954b. On accessory chromosomes in *Festuca pratensis*. II. Inheritance of the standard type of accessory chromosomes. Hereditas, Lund. *40*: 425–437.
———1956a. On accessory chromosomes in *Festuca pratensis*. III. Frequency and geographical distribution of plants with accessory chromosomes. Hereditas, Lund. *42*: 189–210.
——— 1956b. On accessory chromosomes in *Festuca pratensis*. IV. Cytology and inheritance of small and large accessory chromosomes. Hereditas, Lund. *42*: 235–260.
——— 1956c. Cytogenetics of accessory chromosomes in *Phleum phleoides*. Hereditas, Lund. *42*: 443–466.
——— 1957a. On accessory chromosomes in *Festuca pratensis*. V. Influence of accessory chromosomes on fertility and vegetative development. Hereditas Lund. *43*: 211–235.
——— 1957b. Further studies on accessory chromosomes in grasses. Hereditas Lund. *43*: 236–297.
Cleland, R. E. 1951. Extra, diminutive chromosomes in *Oenothera*. Evolution *5*: 165–176.
Darlington, C. D. 1956a. Natural populations and the breakdown of classical genetics. Proc. R. Soc. Ser. B. Biol. Sc., Lond. *145*: 350–364.
——— 1956b. Chromosome Botany. London: Allen and Unwin.
Darlington, C. D. and P. T. Thomas. 1941. Morbid mitosis and the activity of inert chromosomes in *Sorghum*. Proc. R. Soc. Ser. B. Biol. Sc., Lond. *130*: 127–150.
Darlington, C. D. and M. B. Upcott. 1941. The activity of inert chromosomes in *Zea mays*. J. Genet., Cambr. *41*: 275–296.
Ehrendorfer, F. 1957. Akzessorische Chromosomen, Kreuzungsfertilität und Polyploidie beim *Achillea millefolium*-Komplex (*Compositae*). Naturwissenschaften *44*: 405–406.
Fernandes, A. 1949. Le problème de l'hétérochromatinisation chez *Narcissus bulbocodium* L. Bol. Soc. broteria. *23* (II Série): 5–88.
Fröst, S. 1956. The cytological behaviour of accessory chromosomes in *Centaurea scabiosa*. Hereditas, Lund. *42*: 415–431.
——— 1957. The inheritance of the accessory chromosomes in *Centaurea scabiosa*. Hereditas, Lund. *43*: 403–422.
——— 1958a. Studies of the genetical effects of accessory chromosomes in *Centaurea scabiosa*. Hereditas, Lund. *44*: 112–122.
——— 1958b. The geographical distribution of accessory chromosomes in *Centaurea scabiosa*. Hereditas, Lund. *44*: 75–111.
Hasegawa, N. 1934. A cytological study on 8-chromosome rye. Cytologia *6*, (1): 68–77.
Håkansson, A. 1948a. Behaviour of accessory rye chromosomes in the embryo-sac. Hereditas, Lund. *34*: 35–59.
——— 1948b. Embryology of *Poa alpina* plants with accessory chromosomes. Hereditas, Lund. *34*: 233–247.
Janaki-Ammal, E. K. 1940. Chromosome diminution in a plant. Nature, Lond. *146*: 839–840.
Kayano, H. 1956a. Cytogenetic studies in *Lilium callosum*. I. Three types of supernumerary chromosomes. Mem. Fac. Sc., Kyushu Univ., Ser. E. (Biol.) *2* (2): 45–52
——— 1956b. Cytogenetic studies in *Lilium callosum*. II. Preferential segregation of a supernumerary chromosome. Mem. Fac. Sc., Kyushu Univ., Ser. E. (Biol.) *2*(2): 53–60.
——— 1957. Cytogenetic studies in *Lilium callosum*. III. Preferential segregation of a supernumerary chromosome in EMCs. Proc. Jap. Acad. *33* (9): 553–558.
Lewis, H. 1951. The origin of supernumerary chromosomes in natural populations of *Clarkia elegans*. Evolution *5*: 142–157.
——— 1953. Quantitative variation in wild genotypes of *Clarkia*. I.U.B.S. Symposium on Genetics of Population Structure. Pavia 1953, pp. 114–125.
Longley, A. E. 1927. Supernumerary chromosomes in *Zea mays*. J. Agr. Res. *35*: 769–784.
——— 1956. The origin of diminutive B-type chromosomes in maize. Am. J. Bot. *43*: 18–22.
Melander, Y. 1950. Accessory chromosomes in animals, especially in *Polycelis tenuis*. Hereditas, Lund. *36*: 19–38.
Milinkovic, V. 1957. Accessory chromosomes in the roots of *Poa alpina*. Hereditas, Lund. *43*: 583–588.
Müntzing, A. 1943. Genetical effects of duplicated fragment chromosomes in rye. Hereditas, Lund. *29*: 91–112.
——— 1946a. Different chromosome numbers in root tips and pollen mother cells in a sexual strain of *Poa alpina*. Hereditas, Lund. *32*: 127–129.

——— 1946b. Cytological studies of extra fragment chromosomes in rye. III. The mechanism of non-disjunction at the pollen mitosis. Hereditas, Lund. *32*: 97–119.
——— 1948. Accessory chromosomes in *Poa alpina*. Heredity *2*: 49–61.
——— 1949. Accessory chromosomes in *Secale* and *Poa*. Proc. VIII Internat. Congr. Genet., Hereditas, Lund. (Suppl.), pp. 402–411.
——— 1954. Cyto-genetics of accessory chromosomes (B-chromosomes). Proc. IX Internat. Congr. Genet. Caryologia, (Suppl.) *6*: 282–301.
——— 1957. Frequency of accessory chromosomes in rye strains from Iran and Korea. Hereditas, Lund. *43*: 682–685.
MÜNTZING, A., and A. NYGREN. 1955. A new diploid variety of *Poa alpina* with two accessory chromosomes at meiosis. Hereditas, Lund. *41*: 405–422.
NYGREN, A. 1957. *Poa timoleontis* Heldr., a new diploid species of the section *Bolbophorum* A. & Gr. with accessory chromosomes only in the meiosis. Kungl. Lantbrukshögskolans Ann., *23*: 489–495.
OESTERGREN, G. 1945. Parasitic nature of extra fragment chromosomes. Bot. Notiser *1945*: 157–163.
——— 1947. Heterochromatic B-chromosomes in Anthoxanthum. Hereditas, Lund. *33*: 261–296.
RANDOLPH, L. F. 1928. Types of supernumerary chromosomes in maize. (Abstract) Anat. Rec. *41*: 102.
——— 1941. Genetic characteristics of the B chromosomes in maize. Genetics *26*: 608 631.
REESE, G. 1954. Euploidie, Aneuploidie und B-chromosomen bei *Caltha palustris* L. Planta *44*: 203–268.
RESENDE, F. and P. DA FRANCA. 1946. Sur l'origine de nouvelles formes, II. Portug. acta biol. ser. A. *1* (3): 289–307.
ROMAN, H. 1947. Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. Genetics *32*: 391–409.
——— 1948. Directed fertilization in maize. Proc. Nat. Acad. Sci. U.S. *34*: 36–42.
RUTISHAUSER, A. 1956a. Genetics of fragment chromosomes in *Trillium grandiflorum*. Heredity *10*: 195–204.
——— 1956b. Chromosome distribution and spontaneous chromosome breakage in *Trillium grandiflorum*. Heredity *10*: 367–407.
SORSA, V. 1956. The quadripolar spindle and the change of orientation of the chromosomes in meiosis of *Sphagnum*. Ann. Acad. Sci. Fenn., Ser. A. IV. Biol. 33. Pp. 64.
STEVENS, N. M. 1908. The chromosomes in *Diabrotica vittata*, *Diabrotica soror* and *Diabrotica* 12-*punctata*: A contribution to the literature on heterochromosomes and sex determination. J. Exp. Zool. *5*: 453–470.
VAARAMA, A. 1953a. Chromosome fragmentation and accessory chromosomes in *Orthotrichum tenellum*. Hereditas, Lund. *39*: 305–316.
——— 1953b. Some chromosome numbers of Californian and Finnish moss species. Bryologist *56* (3): 169–177.
VIRKII, N. 1954. Akzessorische Chromosomen bei zwei Käfern, *Epicometis hirta* Poda und *Oryctes nasicornis* L. (Scarabaeidae). Ann. Acad. Sci. Fenn., Ser. A, IV. Biol. 26, pp. 1–19.
WITKUS, E. R., T. J. LOWERY, and C. A. BERGER. 1955. The cytology of *Xanthisma texanum* D.C. II. Additional chromosome types. Bull. Torrey Bot. Club *82*: 367–376.

GENETICS AND THE DESTINY OF MAN

Theodosius Dobzhansky[1]

SCIENCE is a form of esthetic endeavor; scientific discoveries may be as beautiful as works of art. To illustrate this point I should, perhaps, have confined myself to exposition of some of the masterworks of classical genetics. I elect instead to discuss topics concerning which speculation abounds and positive knowledge is disconcertingly meager. For is there a problem more perplexing, and yet more inescapable, than that of human destiny? Moreover, I believe that the process of scientific inquiry is as fascinating as its results. Life consists in striving, and is meaningless without it.

The human body starts its existence as a fertilized egg cell, weighing about one–twenty millionth of an ounce. An adult is some fifty billion times heavier. The growth occurs through assimilation of food. Quite literally, man is a conglomeration of transformed groceries. And yet, this conglomeration is alive, feels joy and suffering, is conscious of self, and of the existence of other persons and of the universe. In the words of one of the Dead Sea Scrolls: "So walk I on uplands unbounded, and know that there is hope, for that which Thou didst mold out of dust to have consort with things eternal."

How is this possible? How can groceries be transformed so as to come alive, and even hope to "consort with things eternal"? The nearest approach to an answer is that this transformation is brought about by the action of the genes which man has inherited from his ancestors. Chemical geneticists find that the genes are composed of a remarkable class of substances—deoxyribose nucleic acids associated with proteins—and that the genetic specificity, the genetic information, resides in the nucleic acids. Now, genes come only from genes. A gene somehow replicates itself, constructs its own copy from materials in its environment, ultimately from food. Self-reproduction is the basic property of life; we speculate that the first gene was also the first life. Those who take inordinate pride in the antiquity of their family lineages should be reminded that every man, and indeed every worm, is a direct descendant of the primordial protoplasm itself, which lived two billion or more years ago.

A century ago, in 1858, the theory of evolution was announced by Darwin and Wallace. All forms of life, including man, are related by common descent, but whatever the primordial genes may have been, our genes are surely different. To a modern geneticist, evolution means that the genes underwent many changes, mutations, of their structure. The number of genes in a human sex cell is surmised to be of the order of tens of thousands, and each of these genes must be a product of eons of evolutionary development by successive mutations. Our biological patrimony, the human genetic equipment, man's genotype, has been shaped step by step, and gradually perfected, in the course of its evolutionary history. It is owing to this historical process that the genes which compose our genotype are articulated in a harmonious system. In suitable environments, human genes engender the development of a body which is a masterwork of orderly complexity and of apparent purposefulness.

[1]Department of Zoology, Columbia University, New York, N.Y., U.S.A.

We are confronted, however, with a fact which may seem to shake the theory of evolution by summation of mutational steps to its very foundations. Mutants observed in laboratories and experimental fields are usually detrimental to the organism. Many can be described as hereditary diseases or monstrosities, some are downright lethal, and others cause milder weaknesses or disabilities. The mutant Drosophila flies, which have served for almost half a century as materials for teaching genetics to biology students, are obviously a collection of freaks and cripples. Surely, changes of this sort could only result in degeneration, not in adaptation, of the organism to its environment. Here I can attempt no more than to indicate the broad lines of the argument which resolves the paradox of adaptive evolution issuing from apparently decadent mutants.

The argument is twofold. First, the statement that a mutation is injurious or useful can only be meaningful if the environment in which the mutant lives is specified. An organism is fit or unfit with respect to its environment. Secondly, evolution is not simply summation of mutational steps, it is accumulation of useful mutants. Only a tiny fraction of the mutants which arose in man's ancestry have eventually become constituents of the human genotype. Darwin concluded that hereditary variability becomes evolution if and when it is oriented by natural selection. In the language of genetics, mutation and sexual reproduction supply merely the raw materials from which evolutionary changes are shaped by natural selection. Evolution is a response of living matter to environmental opportunity. But the environment does not alter the genotype directly as Lamarckists and a reactionary school which for a time wielded dictatorial powers over Russian biology wrongly thought. Environment presents challenges of opportunity, to which the organism may or may not respond by evolutionary alterations. Whether it does or does not depends upon the availability at the proper time and place of genetic variants which survive and reproduce in the new environments as well as or better than the ancestral type is capable of doing. Evolution is the outcome of a triangular encounter between environment, mutation, and selection.

As in Drosophila flies or in bacteria, mutants arise in man quite regardless of whether they may or may not be useful. In fact, most of them are harmful. But if the mutation process were suppressed evolution would eventually come to a halt. Selection favors the genetic endowments which make their possessors reproductively fit in existing environments. And yet, natural selection lacks foresight; it is as incapable at present of maintaining the genotypes which were useful to men of the Stone Age as it is powerless to evoke changes which will be helpful in the future Atomic Age. Only man could, if he so wished, supply the foresight which nature has failed to provide in evolution. To do so, it will be necessary to substitute a planned artificial selection for the unplanned natural one.

One aspect of this problem, and perhaps not the most important one, has in recent years engaged much public attention. This is the genetic damage which human populations are liable to suffer owing to the use and misuse of atomic energies. Some of the relevant facts are almost deceptively simple—deceptively because this very simplicity tends to gloss over serious gaps in our understanding. In 1926, H. J. Muller discovered that mutants appeared more often in the progenies of Drosophila flies treated with X-rays than in untreated controls. Other investigators found X-rays mutation-inducing, or mutagenic, in all organisms which lend themselves to appropriate experimentation. Man is evidently not such an organism, but X-rays are beyond reasonable doubt mutagenic also in man. All so-called high-energy radiations, from softest X-rays to gamma rays, and presumably to cosmic rays, are mutagenic.

Furthermore, the incidence of induced gene mutations is simply proportional to the amount of the radiation reaching the sex cells. Therefore, as far as the genetic damage is concerned, there is no such thing as a safe or permissible amount of radiation. Physicists measure the radiation in so-called r-units, which refer to the numbers of ionizations produced by the passage of the rays through a body. Now, a given number of r-units, no matter how small, will cause a proportionate genetic damage. The damage is independent of whether the radiation is received in a single burst, from an atomic explosion, or gradually over years or decades, from absorbed fallout products. This simple proportionality of the incidence of mutations to the radiation applied has been questioned, especially by non-geneticists. Indeed, the amounts of spontaneous radiation omnipresent in nature, owing to cosmic rays and to radioactive isotopes, are, except under special circumstances, of the order of 3 r-units per thirty years, the age which about corresponds to the middle of the reproductive life in man. Experiments on Drosophila have been made with from 25 to 8,000 r-units, and on some bacteria from 8 r-units upwards. With smaller dosages the experiments become prohibitively difficult. It is fair to say, however, that the direct proportionality rule describes the existing information so well that the burden of proof lies on him who would doubt it.

Our confidence vanishes when we ponder the fate of the mutants after they have been produced. Thus far, almost the entire attention of investigators has been concentrated on finding out how many mutations radiation induces. It can hardly be overstressed that we know woefully little about what happens to the mutants after they enter the genetic endowment of a living sexual population. True enough, mutants the effects of which are easily studiable produce at least some losses of fitness in the environments in which the species normally lives. Radiation-induced mutants are, if anything, more insidious and crippling than spontaneous mutants. And yet the pioneer work of Gustafsson on barley, recently extended to other agricultural plants by several workers, has shown that some induced mutants increase the yield, at least when heterozygous. Radiation may turn out to be a powerful tool in breeding work. Agricultural plants are cultivated in environments quite different from those in which their ancestors lived; mutants useful to the agriculturist may be injurious to the plants if they lived in the state of nature.

Perhaps most regrettable is the dearth of information concerning the influence on fitness of mutants, with individually small effects, of the so-called polygenes. Sharp discontinuous mutants in *Drosophila* and in man make beautiful examples of Mendelian inheritance, but their importance in evolution is relatively limited. Such traits as stature, weight, longevity, susceptibility to disease, and skin-, hair-, and eye-colors in man, yield of milk, meat, eggs, grain, or the living mass in agricultural animals and plants, are conditioned mostly by gene complexes which are hard to analyze into separate gene components. Timofeeff-Ressowsky and Kerkis have independently shown more than two decades ago that X-ray treatments induce detrimental polygene changes in *Drosophila*. At first glance, this may seem to contradict the brilliant recent work of Bruce Wallace. Wallace's material consists of *Drosophila* flies which have been made, by means of special crosses, to carry two identical chromosomes of a certain pair. Such flies normally suffer a loss of vigor compared to normal outbred flies. Now, Wallace finds that the presence in such flies of one chromosome treated with X-rays gives a slight, but significant, improvement of their viability. Has Wallace shown that it is good for a population to be exposed to X-rays? Not at all! But what he has shown is important. It is that, under conditions of his experiments, the X-ray induced mutants are *on the average* increasing the viability of the flies when present in single dose, in heterozygous

condition. There is good evidence that the same mutants would yield impaired viabilities when in double dose, in homozygous condition.

The existence of adaptively ambivalent mutants poses serious problems which only future investigations may solve. We do not know how frequent such mutants are in *Drosophila* populations, not to speak of human populations. If they are rare, then the ideal state of a population may perhaps be genetic uniformity, and the presence of mutant genes would be simply a genetic load or a genetic burden, which we must try to minimize. But it may turn out that genes which produce hybrid vigor, or heterosis, are very frequent, and that our biological welfare depends upon our being multiple heterozygous. Surely, these are not alternative possibilities, and the adaptive optimum for man may be some intermediate state. I think that the best course is to admit that our understanding of the genetics of human populations is very sketchy, and it is most unwise to give an appearance of simplicity to this singularly complex matter.

Lest the above arguments be misinterpreted, I wish to state as emphatically as I can that they do not undermine the basic principle that any increase of mutation rates in human populations means an added increment of human suffering. The exposure of people to mutagenic radiations should be reduced to an unavoidable minimum. And this applies to exposure from fallout products of testing of atomic weapons, to X-rays employed in medical practice, and to the radiations to which peaceful uses of the atomic energies will inevitably subject increasing numbers of persons. Many of the discussions of the genetic risks from radiations, even by eminent scientists, display a curious aura of misplaced concreteness. Computations are made to show how many thousands of genetically handicapped persons will be born over many generations as a result of exposure to the radiation from fallout products. Yet some scientific pundits try to comfort us with the cheerful thought that this anguish and wretchedness will simply be superadded to the even greater amounts of misery which will arise from mutations not caused by the radiation effects. Testing of atomic weapons is said to be indispensable for preservation of freedom, hence sacrifice of thousands of innocents is justified. After all, in wartime generals and admirals send millions to their death. But should we not ask, is it right to doom a single human being for the benefit of others? And is not the really fundamental question whether preservation of freedom can be entrusted to those who would make and use atomic weapons?

Anyway, the problem of genetic damage by radiation should be viewed in its proper perspective. In man, biological evolution has transcended itself. It has equipped mankind with genotypes which have rendered possible the origin and evolution of culture. Culture is an adaptive instrument of immense potency; species other than man become adapted to their environments by changing their genes, man changes his environments to conform to his genes. In man the evolution of life has reached a turning point; culture is superorganic, and cultural evolution has laws of its own. But it must not be forgotten that the superorganic has an organic basis, and neither human culture nor human genes are unalterable.

I think that we must definitely reject the notion that mankind has not changed genetically to any appreciable extent since the inception of the human cultural phase, one hundred thousand or more years ago. Human genes have been continuously patterned and re-patterned by natural selection, precisely because they made possible adaptation to the environment by means of culture. They bestowed upon our species the ability to communicate by means of symbolic language. Human genes have ushered in an increasing refinement of what Pavlov has termed the second signal system, concerned with symbol formation and symbolic thought. The

genetic basis of culture is the greatest achievement of biological evolution, and it is utterly improbable that it can remain static for any length of time.

This poses a problem of incalculable significance, of understanding and eventually of controlling the processes of change which affect the genetic endowment of mankind, on which the biological as well as the cultural prospects inexorably depend. Faced with this problem a biologist is in an awkward position—he knows too much to ignore it, and not enough to solve it. For one of the paradoxes of this age of science is that man has applied his burgeoning knowledge to almost everything but himself. Does human evolution flow like a river, ever onward despite a meandering course? We cannot be completely sure as yet. But one thing is certain—with the advance of culture natural selection promotes genetic endowments which are different in some respects from those which selection favored in our pre-human ancestors.

This is commonly illustrated by reference to the progress and accomplishments of the art of healing. Tuberculosis was until recently one of the major scourges of humanity and an inveterate killer of persons of reproductive and pre-reproductive ages. Modern medicine has all but eliminated tuberculosis in some countries, and bids fair to do so universally. Control of malaria throughout the tropics may prove an even more outstanding achievement. Now, there is reasonably good evidence that susceptibility to tuberculosis, and also to malaria, is genetically conditioned. I hasten to add that discovery of genetic conditioning does not refute the existence of an interacting environmental conditioning. This is proven by the very fact that the diseases in question can be eliminated by improved hygiene and sanitation. We can infer, then, that the genotypes which predispose people to tuberculosis and malaria are eliminated by selection less often at present than they used to be. A reasonable further inference is that these genetic constitutions will grow in frequency in human populations.

Natural selection has, no doubt, become relaxed with respect to quite a number of genetic traits which were dangerous or fatal under conditions of a not so remote past. It takes no prophetic gift to predict that this relaxation will continue in the future. But it does not follow that natural selection no longer operates in the human species; nor is there any need to conjure an apocalyptic vision of impending doom, which some writers have fabricated. Let it not be forgotten that natural selection is not a *deus ex machina* but an automatic consequence of the encounters between a living population and its environment. As pointed out above, it makes no sense to talk of a genotype being favorable or unfavorable if the environment is not specified. The environment of the Machine Age is not the same as that of the Stone Age. It can hardly be expected that natural selection will favor the same genetic constitutions perpetually. Moreover, this instability of selection is nothing peculiar to man or to the civilized state; natural selection shuffled the genes of free-living animals and plants differently during the Ice Age and in post-glacial times. As the environment changes, selection may become more stringent in some respects and less so in others.

There is, indeed, some evidence that for certain human genes natural selection is more rigorous at present than it was in the past. The clearest example is the gene for porphyria studied in South African populations by Dr. Dean. Its carriers can be identified by a simple chemical test; if they are country folks living under rustic conditions they suffer no major inconvenience; however, certain drugs which are non-toxic to most people, such as some sleeping pills and pain killers, cause grave and even fatal illness in porphyrics. The invention of environments which contain sleeping pills must certainly be credited to the medical profession, and many of us

tender our thanks for this discovery. But the carriers of porphyria are decidedly unfit in these environments.

There are reasons to think that the environments of the Machine Age discriminate against many other genetic constitutions. Take, for example, the suggestive fact that epidemics of paralyzing polio occur chiefly in countries with high standards of sanitation, but not in those with low standards, perhaps because in the latter almost everybody acquires an immunity through subclinical infection. The evidence for genetic control of the susceptibility to polio is far from convincing, but taking it at face value we would have to conclude that the relative fitness of some genotypes is decreased by sanitation. Consider also that shortages of food were the greatest peril to man's life until the dangers of overeating became more menacing to some "chosen" people. Now, one may surmise that some genetic constitutions which are able efficiently to utilize and store up nourishment are also prone to obesity, high cholesterol levels in the blood, and heart disease. Prosperity may put them at a relative disadvantage. Consider finally that living in what are sometimes referred to as "ulcer belt" cities may be hard on many genetic constitutions which thrive on pastoral simplicity.

Natural selection is at work in modern mankind, although it functions in what, in a manner of speaking, are highly unnatural environments. We must, however, be on our guard not to be led astray by this treacherous adjective "natural." In modern genetics, selection refers simply to the fact that in a population composed of a variety of genotypes, some of the latter leave in succeeding generations greater numbers of progeny relative to other genotypes. The heroic, or awesome, image of the surviving "fittest," so dear to social Darwinists and racists, is degraded to the rather unromantic figure of a parent or grandparent of a prolific family. When family planning will be governed by an intent to perpetuate certain genotypes and to limit the perpetuation of others, mankind will have reached the era of artificial selection. In our time natural selection is in the saddle. That this selection now favors different genetic endowments than it did in the Cave Man is patent. Even before the advent of the Machine Age, not to speak of the Atomic Age, man was irrevocably committed to live in environments created by his culture. In this respect, man passed the point of no return many thousands of years before he knew it.

Fossil men had large and strong teeth, but their crowns were much worn at relatively young ages. Cooked foods which we eat are less abrasive, yet all the same, some of us have to resort to wearing false teeth. Parenthetically, we may note that tooth caries tormented also some prehistoric men. But we live in environments in which dentists abound, and this augments the fitness of genetic endowments which are liable to yield weak teeth. Surely, it does not follow that for men of the Machine Age it is desirable to have weak teeth; health authorities do their best to improve the teeth in the growing generation. But natural selection has taken other paths in civilized man; the selection for the maintenance of strong teeth has become relaxed in comparison with bygone ages.

The example of teeth and dentists is a fair illustration of a much more general principle. It is a fallacy to think that natural selection necessarily promotes genotypes which are good or desirable in our human estimation. In fact, natural selection cannot be relied upon even to preserve the species from extinction. The evidence of this is plentiful—paleontological museums are full of fossilized remains of creatures most of which died out without issue. Yet their evolution was under control of natural selection; it occurred under unadulterated "natural" conditions. This is an only apparent paradox. Extinction is a result of the opportunism of natural selection —selection may drive a species into a blind alley of specialized adaptation to an

environment which does not last. Man can rely even less upon natural selection. Our ideas of what is desirable and undesirable, superior and inferior, good and bad, are fashioned by the evolution of culture. They are not bestowed upon us by our genes. The myth of limitless perfectibility of man, this cherished heritage of the Age of Enlightenment, is not encompassed by the theory of natural selection.

All this does not mean that biologists should abdicate their responsibility for the direction of human evolution—and I mean biological as well as cultural evolutions, since they are interdependent. I am persuaded that precisely the opposite conclusion is warranted. Since man cannot rely upon biological forces to keep him at peace with his own nature and with his environment, he can depend only on his wisdom and knowledge, including knowledge of biology and genetics, to help him accomplish this end. Perhaps the frenetic confusion of recent years concerning the genetic hazards of atomic energy will prove useful after all. The peaceful uses of atomic energy are with us to stay, and the debates about their hazards may help to direct the attention of people to the need of more knowledge and understanding of man and of his genetic substratum. For at least two centuries the frontiers of human knowledge were advancing most rapidly in physical sciences and in related technology. Biology was and still is a poor relative. What of the future? Although the future often bears only a faint resemblance to bold prophecies, it does look as if we are about to enter a period when biology will take the lead. Man can hardly afford to be in the dark concerning his destiny much longer.

Some two and a half centuries ago, Blaise Pascal wrote these immortal lines: "When I consider the short duration of my life, swallowed up in the eternity before and after, the little space which I fill, and even can see, engulfed in the infinite immensity of spaces of which I am ignorant, and which know me not, I am frightened, and am astonished at being here rather than there; for there is no reason why here rather than there, why now rather than then. . . . The eternal silence of these infinite spaces frightens me." What light can genetics throw on human destiny? It makes us less ignorant of the immensity of the infinite spaces. Their silence becomes less frightening.

Our species, mankind, is a single genetic unit. The atomic bombs which exploded over the cities of Hiroshima and Nagasaki have induced mutations which will not be confined to the populations of these two cities. They will be added to the genetic load of the population of Japan and ultimately of the world. When a problem like that of the genetic damage likely to be caused by radiations is considered, nobody can rest satisfied if only he himself has escaped radiation exposure. The future of my genes depends ultimately on that of everybody else's genes. Genetics has vindicated the great words of John Donne: ". . . any man's death diminishes me, because I am involved in mankind."

Western civilization is rapidly acquiring the character of universality. This fact imposes upon scientists a heavy responsibility. For mankind will from now on be the molder of its own destiny, and perhaps of the destiny of all life in the Universe. In Man, the process of evolution has created its own directing center. But direction requires knowledge and wisdom. I can hardly do better than to conclude this lecture by quoting Teilhard de Chardin: "Taken in the full modern sense of the word, Science is the twin sister of Humanity. Born together, these two ideas, or two dreams, have grown together, until they attained a quasi-religious value during the last century. They have also suffered the same dishonors. Which does not prevent them, supporting each other, from always representing the ideals to which our imagination comes every time it tries to materialize on earth the reasons for its faith and its hope."

GENETICS, THE GENE, AND THE HIERARCHY OF BIOLOGICAL SCIENCES[1, 2]

Sewall Wright

"HISTORY shows that throughout the centuries . . . natural history constitutes the perennial rootstock or stolon of biologic science and that it retains this character because it satisfies some of our most fundamental and vital interests in organisms as living individuals more or less like ourselves. From time to time the stolon has produced special disciplines which have grown into great, flourishing complexes. . . . More recently another dear little bud, genetics, has come off, so promising, so self conscious, but alas, so constricted at the base."

This quotation from William Morton Wheeler (1923) expresses a common viewpoint of non-geneticists of Wheeler's time. There were even geneticists who saw little future in genetics, not because its field seemed trivial, but rather because they were so dazzled by the achievements of the first two decades of the century that they felt that there was little left for them to do. I recall a talk I had about that time with a prominent geneticist who had decided to go into a different field. "Genetics," he told me, "is a cow that has been milked."

There are two polar interests in biology. One is the fascination with the diversity of life which Dr. Wheeler so notably exemplified. I can testify to the enthusiasm which he could arouse by his lectures and the exhilaration which one felt during field trips conducted by him. At the other pole is the fascination with the interconnectedness of things. My thesis is that genetics has become the stolon, in a different sense from Wheeler's, which is most effectively binding the whole field of biology into a unified discipline that may yet rival the physical sciences.

THE CLASSIFICATION OF THE BIOLOGICAL SCIENCES

There are many ways in which one may classify the biological sciences. I will not pause on classification by utility—medicine and public health, the agricultural sciences, forestry, etc.—though genetics has much to contribute to these. Nor will I pause on classification by kind of organism—mammalogy, ornithology, and so on down. Genetics cuts across them all. Branches of science also tend to grow out of techniques. Genetics centers in the techniques of breeding organisms. Breeding has turned out to be a remarkably penetrating technique, and one capable of joining forces with other techniques of the most diverse sorts in fruitful ways. For our purpose, however, the most instructive classification is by principles (Wright, 1953).

Biology deals with a hierarchy of entities. The primary subdivision of biology is perhaps best made according to level in this hierarchy. Biology began with *individuals*, Wheeler's natural history. It worked down through *organs* and *tissues*, categories that cut across each other, to the *cell*, as the almost universal unit of

[1]Presidential Address, X International Congress of Genetics, Montreal, August 20, 1958.
[2]Paper no. 720 from the Department of Genetics, University of Wisconsin, Madison, Wisconsin, U.S.A.

TABLE I

CLASSIFICATION OF THE BIOLOGICAL SCIENCES*

Biological level		Climax phase: Description	Climax phase: Dynamics	History: Description	History: Dynamics	Multiplication
Ecologic system	World biota	Biogeography	Biotic stability	Paleontology	Biotic evolution	
	Local biota	Ecology (community)		Ecologic succession		
Interbreeding population	Species	Taxonomy	Species stability	Phylogeny	Macroevolution Transformation	Species cleavage
	Deme	Demography (7) *Population genetics*			Microevolution (7) *Population genetics*	
Multicellular organism	Individual	External characters	Behavior (6) *Genetics of behavior*	Life history		Physiology of reproduction (1) *Formal genetics*
	Organ	Anatomy	Gross physiology	Descriptive embryology	Morphogenesis (5) *Developmental genetics*	
	Tissue	Histology			Histogenesis	
Cell	Cytoplasm and nucleus	Cytology	General physiology (4) *Physiological genetics*	(2) *Cytogenetics*		Mitosis
Autonomous macromolecule	Gene DNA	*Gene chemistry*	(3) *Theory of the gene* *Gene physiology*	*Gene mutation* *Description*	*Process*	*Gene duplication*
Non-autonomous molecule		Biochemistry				

*The locations of branches of genetics are indicated in italics.

structure and function, and one that behaves in significant ways as an autonomous organism on its own account. For many years, biologists thought of the cell as the ultimate unit of life, composed of ordinary non-living molecules but organized in a way that conveys the properties of life. The geneticist has added a new level, the *gene*, an entity present in large numbers in the cell, controlling the specific properties of the cell but itself autonomous with respect to its specificity.

Moving in the other direction from the individual, towards larger units, we may proceed through local interbreeding populations (*demes*) to the *species*, an entity that persists with certain properties in spite of frequent complete replacement of all of its constituents. The integrating principle of the species is biparental reproduction. Cutting across this, somewhat as organs cut across tissues, are *ecologic systems* which maintain more or less constant patterns by means of self-regulatory

interactions among the local representatives of many species. Finally, we have the entire *fauna* and *flora* of the world as a single, interconnected, more or less persistent system, a vast symplasm, to use Wheeler's word.

At all levels there are problems: (1) of describing the persistent entities and (2) of accounting for their persistence; (3) of describing and (4) accounting for the orderly second-order changes by which biologic entities characteristically develop; and finally (5) of describing and interpreting the processes by which the entities at the lower levels multiply.

Genetics at the Level of the Individual

Genetics began at the level of the individual with curiosity about the similarities and differences among individuals related by descent. Rapid exploration in many species of animals and plants, once the key was given, led to the generalization that the rules of inheritance are everywhere much the same. Genetics, therefore, settled down largely to the intensive study of a few especially favorable higher organisms: for example, *Drosophila*, the silkworm, the mouse, and the domestic fowl among animals; the garden and sweet pea, maize, antirrhinum, cotton, and tobacco among plants.

In the last few years, however, our concepts have been greatly enriched by a tremendous development of the genetics of micro-organisms, some of which could not even be crossed until recently: *Paramecium*, *Neurospora*, *Aspergillus*, yeast, various bacteria and their phages, and others.

I believe that the list of organisms that can contribute significantly is not yet exhausted. Many of those that have been studied less extensively have contributed interesting but often overlooked phenomena, not encountered in the major objects of study. I am thinking of such things as the very odd genetics found by Metz in *Sciara* (1938). There is still a tremendous field of comparative genetics that will reward pioneers.

Genetics and Cytology

Mendel's laws of heredity and a knowledge of the behavior of the chromosomes in mitosis and meiosis first entered the same minds very shortly after 1900. The firm establishment of the chromosome theory of heredity was the great achievement of the next two decades. Cytology became the first major branch of biology with which genetics became welded, the field of cytogenetics.

In the middle period of our history since 1900, the discovery of modes of inducing gene and chromosome mutations, and the demonstration of one-to-one relations between details of the linkage maps and those of the salivary chromosomes of *Drosophila melanogaster*, were among the outstanding events at this cytogenetic level.

Recently, the electron microscope has provided means for carrying the analysis of chromosomes much farther. The threadlike leptotene chromosomes as seen with the highest powered light microscope, readily suggested the notion of single giant molecules. The tropical jungle of twisting lianas which the electron microscopists present as the real appearance of these same objects, reminds us that the chromosome is separated from even macromolecules by several orders of magnitude (Kaufmann and McDonald, 1956; Ris, 1957). The Watson-Crick molecule of DNA is some 20Å in diameter. The numerous chromosome fibrils, revealed in electron-micrographs, seem to be some ten times this in diameter, and the finest threads

distinguishable under the light microscope are some ten times again as thick. The relation between a DNA molecule and a leptotene chromosome is thus somewhat like that between a wire 1 mm. thick and a cable some 10 cm. thick.

There are obviously still plenty of problems in cytogenetics. With the new techniques, including radioactive markers (Mazia and Plaut, 1955; Taylor, Woods, and Hughes, 1957), as well as the electron microscope, there are exciting prospects.

The Gene: Earlier Ideas

We come now to the level in the biological hierarchy that is the distinctive contribution of genetics, the gene. It was easy enough when I first used this two-dimensional classification of the biological sciences some forty years ago to subdivide the bottom row, the theory of the gene, into formal categories, similar to those at the higher levels, but these had little substance, and it was hard to imagine that they ever would have much. The recent period of our history has, however, been characterized especially by giving substance to just these aspects of genetics. On the other hand, the disconcerting question, whether there is any such thing as a gene, has been raised by Goldschmidt (1946), a very great geneticist, whose provocative points of view we are going to miss badly. I wish to devote most of my time to questions at this basic level.

For his dominant and recessive units Mendel used the word *Merkmal*, which Bateson translated into English as character. The term unit character seemed to imply a return to the discredited preformation theory of heredity and doubtless contributed to the suspicion of genetics felt by other biologists. It was, however, rapidly established that the heredity of characters tended to split up into many independently segregating pairs. It came to be recognized that unit factor was a better term than unit character and, a little later, Johannsen's term "gene" was welcomed as a distinctive one for the basic unit of heredity.

There has remained some ambiguity in the frequent use of this term for both the common substrate of alternative units and a particular one of these. I will use the later term, "locus," for the substrate and restrict "gene" to a particular representative of a locus, considered by itself.

The fact that analysis seemed to indicate the existence of dominant and recessive pairs led to the presence and absence hypothesis of Bateson and Punnett. As early as 1904, however, Cuénot, on analyzing the heredity of coat color of the mouse, had clearly demonstrated a set of multiple alternatives, as well as several independent pairs, and it came to be recognized by 1915 that multiple sets were not uncommon.

It was natural at first to extend the presence and absence theory to multiple alleles by postulating differences in quantity of the gene. It was soon realized, however, that qualitative differences in the gene and its primary product, conjectured to be an enzyme, might register physiologically as mere quantitative variations of characters. Then it turned out that pleiotropic effects of multiple alleles did not always fall in the same order. I strained myself considerably at about this time (Wright, 1916) trying to account for lack of parallelism in the effects of four (later five) alleles at the albino locus of the guinea pig on various colors of coat and eye, on the basis of differences in thresholds and competition or its absence. In the end (Wright, 1949) it could only be concluded that the five alleles determined five qualitatively different tyrosinases, even though different thresholds and competition effects were certainly present.

The patterns of differential effect within multiple sets of alleles have indeed turned out to be extraordinarily diverse even though one can usually trace a thread of physiological similarity of some sort. We find complementary (Whiting, 1954; Dunn, 1956) as well as non-complementary recessive alleles of wild type, differences in localization of patterns (Nabours, 1930; Dubinin, 1929; Tan, 1946; Gordon, 1947; Tanaka, 1953), differences in order of effect on different characters that lead in extreme cases to alleles that seem to have no more in common in their differences from type than random non-alleles (such as some of the alleles at the dumpy and spineless loci of *Drosophila melanogaster* (Bridges and Brehme, 1944). There are series in which many antigenic properties occur in all possible relations among the alleles (Irwin, 1947; Stormont, 1955; Wiener and Wexler, 1952): incompatibility, positive or negative correlation, asymmetrical dependence, as well as apparent independence. Finally we have such curious manifestations of qualitative diversity as the self-incompatibility series of many plants (East, 1929; Lewis, 1954) and Whiting's (1943) nine sex-determining alleles of *Habrobracon* (any heterozygote: female; any homozygote or hemizygote: male).

It should be noted, in view of the current confusion in the use of the term allelic, that I am using it to describe a genetic relation, not a physiologic one, in conformity with Bateson's (1909) definition of allelomorphic (of which allelic is a contraction) "alternative to each other in the constitution of the gametes." I am using allele as a representative of a locus, considered in relation to another, irrespective of physiological relations.

Conceptions of the locus as a physical entity were, of course, guided by cytologic observations. The early demonstration of the persistence of individualized chromomeres and their pairing in synapsis (Wenrich, 1916), Belling's demonstration (1928) of as many as two thousand such particles in favorable materials culminating in the demonstration of some five thousand identifiable bands in the salivary chromosomes of *Drosophila* and the location of particular loci in or near such bands by Painter (1934) and Bridges (1935) led to general acceptance of the particulate nature of loci. Estimates of average size of loci in the millions in terms of molecular weight by Morgan (1922) and Muller (1929) seemed to give adequate scope for patterns capable of mutating at hundreds of different sites within a single locus and thus an adequate basis for the complexity of the observed allelic series.

The Chemical Basis of Heredity

Perhaps the most revolutionary achievement of the recent period of our history has involved the chemical basis of heredity. It had been known since Miescher's work in the 1870's that chromosomes (of fish sperm) are largely composed of protein and nucleic acid. Until rather recently, nucleic acid seemed ruled out as the site of specificity by its supposedly monotonous tetranucleotide structure. Most geneticists up to the middle 1940's assumed that there was little choice but to suppose that specificity resided in a pattern of successive amino acids. Then came the discovery that the pneumococcus transforming principle, acting like a free gene, was probably pure DNA (Avery, McLeod, and McCarty, 1944) and the recognition by biochemists that there was after all an adequate basis for specificity in the sequence of nucleotides in chains of indefinitely great length. The demonstration of constancy of DNA per chromosome set (Boivin, Vendrely, and Vendrely, 1948; Mirsky and Ris, 1949) added to the confidence in its genetic significance.

There was a transition period when the conjecture of a reciprocal relation between protein and DNA, each imposing specificity in synthesis of the other (Friedrich-Freksa, 1940), seemed most attractive, but soon Watson and Crick (1953) gave us a model of DNA which indicated a reciprocal relation between two intertwined complementary but oppositely directed strands of this material itself. As this model was based on precise analysis of the X-ray diffraction pattern, it marks a tremendous step beyond mere conjecture toward understanding the actual chemistry of heredity.

This, of course, is only a beginning. The problem of the untwisting of the complementary strands is one that is difficult to contemplate without vertigo, at least if it must proceed through the whole length of the chromonema, with its hundreds of thousands of gyres (Delbrück and Stent, 1957). The difficulty would, of course, be mitigated if the DNA were broken up without disruption of order into units with molecular weights of perhaps 10^6 (some 150 gyres) all capable of untwisting simultaneously.

The problem of how DNA imposes patterns on other macromolecules (RNA, proteins, polysaccharides) has not yet advanced beyond the stage of conjecture. Especially disconcerting is the fact that some viruses have only RNA and protein and no DNA at all.

The problems of the chemical basis of heredity are thus far from solved. An important step has, however, been taken and the nucleic acids, their processes of specific synthesis and of imposition of specific patterns on other macromolecules, have now become the focus of intense effort by biochemists. Genetics and biochemistry have made a contact here that promises to be as fruitful as that of genetics and cytology a generation earlier.

Fine Structure of the Gene

The field that has been investigated most intensively in recent years by distinctively genetic techniques is that of the fine structure of the gene. Present concepts were foreshadowed by the theory of step allelomorphism of Dubinin (1929) and Serebrovsky (1930). Oliver's demonstration (1940) of crossing-over between supposed lozenge alleles in *Drosophila melanogaster* with reconstitution of wild type, and that by Lewis (1945) of position effects associated with crossing-over between star and asteroid, probably located in the components of a double salivary band, initiated a period of successful splitting of many supposedly single loci in this organism. Similar results have been obtained in several other organisms, notably maize (Stadler, 1954; Laughnan, 1955), *Neurospora* (Giles, 1955), *Aspergillus* (Pritchard, 1955), yeast (Roman, 1956), *Salmonella* (Demerec, 1956), and phages (Benzer, 1957; Streisinger and Franklin, 1956).

Interpretation has taken two directions. In some cases, subdivision of a system of supposed alleles with multidimensional variability has seemed to yield components with single types of effect, and thus has led to the hypothesis that heredity is made up, after all, of ultimate particles that behave as units both structurally and physiologically. Some proponents of this view have indeed not hesitated to divide seemingly complex loci into simple components on the basis of physiological effect alone. Unfortunately, thorough analysis of the effects in cases in which complex loci have actually been divided by crossing-over has often not indicated any clear-cut separation into physiologically simple loci (Chovnik and Fox, 1953; Barish and Fox, 1956). It is probable enough that there are unitary patterns in the genic material, whether divisible by crossing-over or not, that determine patterns in a single

macromolecular product, but also that there are a vast number of different sites for mutation and possibilities for multidimensional variability in the activity of this product.

The other interpretation favors complete abolition of the classical structural gene, that is, the view that disruption by crossing-over or by other means, may occur between any two nucleotide pairs of the Watson-Crick molecule. Pontecorvo and Roper (1956), for example, have pointed out that the ratio of amount of recombination (5×10^{-4}) between the outer two of three demonstrated components of the white superlocus of *Drosophila* and the smallest amount between two (8×10^{-6}) is 62, and that a similar calculation from four separable mutations in a superlocus of *Aspergillus* gives 150, suggesting the possibility of almost unlimited subdivision. On the other hand, such facts by no means preclude the possibility that there may be particulate loci varying greatly in length and that the intervals between them may vary greatly in ease of breakage, as is indeed suggested by the very heterogeneous appearance of the salivary chromosomes of the diptera.

The process of actual subdivision of blocks concerned with single physiological processes has gone farthest in studies of phage (Benzer, 1957; Streisinger and Franklin, 1956) and bacteria (Demerec, 1956). From estimates of the total number of nucleotide pairs in the phage, it appears likely that there is no limit to divisibility, short of the nucleotide pair.

Interpretation of these cases and of some cases of apparent rare crossing-over in higher forms has unfortunately been made somewhat doubtful by the recognition (Lindegren, 1953; Mitchell, 1955) of a class of mutations, "conversions," that occur only in heterozygotes, not by crossing-over, although likely to occur in the neighborhood of a crossover. This phenomenon, foreshadowed by a study of a mutable locus of *Drosophila virilis* by Demerec in 1928 and by Winkler's (1930) conversion theory of abnormal tetrad ratios in fungi and mosses, is of great interest in itself as a further inroad on the wastebasket category "mutation." Its occurrence also raises the question whether it is pedantic to use even firmly demonstrated crossing-over at rates characteristic of mutation in defining the boundaries of the basic genetic unit. At rates of 10^{-5} or less per generation between similar mutations, crossing-over itself becomes essentially merely another kind of mutation.

Again it is not clear that recombination in the process of synthesis of naked DNA of phage can be considered as the same phenomenon as crossing-over between homologous chromatids of higher organisms, some two orders of magnitude greater in diameter. Thus, even if phage and bacteria are shown to contain no structural genes, it is quite possible that there has been an evolutionary development of such structures with the several hundredfold increase in the amount of genetic material in higher organisms.

There are some general reasons for suspecting the existence of preferential breakage points in *Drosophila*. Let us assume that the chromonema consists of a succession of physiological units, each involving some thousand nucleotide pairs. If the breakage points of chromosome rearrangements occur at random between nucleotide pairs, there would be only one chance in a thousand that a break would not disrupt a physiological gene and only one in a million that there would be no disruption at either end. Yet 40 per cent of 332 random translocations observed by Patterson and associates (1934) in *Drosophila melanogaster* were viable as homozygotes and over 90 per cent of these were fertile. Moreover, some or all of the damage may well have been due to disruption of interactions between adjacent genes.

Similarly, crossing-over would usually cause at least isoallelic mutation if one supposes that the population carries many isoalleles at high frequencies in each physiological unit. Such mutations may well occur, but an evolution toward greater stability of successful patterns would seem likely, if possible.

In addition to such general arguments, we have the intensive study of Muller (1955) on rearrangements involving a restricted region of the X-chromosome of *Drosophila melanogaster* which indicated a very limited number of breakage points. The study of the Greens (1956) of eighteen lozenge mutations indicated, in very extensive tests, that these fell into just three groups such that crossing-over occurred between but not within them. The studies of David Bonner (1956) and his associates in *Neurospora* may also be referred to here.

We must, however, note the possibility that physiologically significant patterns in the DNA are separated by large inert stretches. The results of Patterson and his associates could be accounted for if there is twice as much inert as active material, even if breakage occurs at random.

Mazia (1954) has found indications that DNA polymers, 4,000 Å long, are bound together longitudinally by Ca or other divalent ions, which present points of relatively easy separation under certain conditions. Kaufmann and MacDonald (1956) have, however, questioned this interpretation of the experimental evidence.

Still another possibility, not necessarily exclusive of either of the preceding, is that there is an evolutionary mechanism for protecting useful sequences of nucleotides from disruption. The identification of Belling's ultimate chromomeres with genes has been questioned on the ground that these are observed to be merely sites of incipient coiling in leptotene (Ris, 1957), but if, as is generally agreed, they occur always at certain definite sites, they must be indicators of real entities. Precocious coiling of definite regions suggests intrachromatid synapsis of tandem duplications of about the period of normal coiling. Perhaps very short tandem duplications have been very much more common in the course of evolution than even Bridges (1935) or Metz (1947) supposed and actually constitute the structural genes by largely restricting breakage to unduplicated intervening regions.

The difficulty may be raised that such duplication would usually interfere with the expression of inactivating mutations. One would, however, expect such duplicants to differentiate, each tending to become inactivated in respects in which the other has retained activity. Moreover, only about 10 per cent of the loci of *Drosophila melanogaster* (if identified with salivary bands) seem capable of conspicuous viable mutation, suggesting that most loci actually are protected from this sort of mutation.

It has long been evident that inversions are also very common in the course of evolution. The result of tendencies toward repeated duplication by unequal crossing-over, toward differentiation of the duplicants, and toward the occurrence of inversions of various lengths, would be subdivision of the chromonema into regions of more or less similar material, bounded by unconformities. One might consider such regions as supergenes or gene clusters, consisting of several or many highly stable genes (that is, single duplications) separable with varying degrees of difficulty according to the amounts of intervening unduplicated material.

While the modes of origin of physiological genes, that is, physiologically significant patterns of nucleotides, and of structural genes are independent on this view, one would expect natural selection to bring about a tendency toward coincidence and to restrict unduplicated material to regions of no physiological significance, in

which disruption, not necessarily at exactly the same level in all strands, would have no physiological consequences.

One of the difficulties of most hypotheses of crossing-over has been the requirement that the exchange points of the two chromatids correspond exactly in position; and this is certainly exacerbated if each consists of numerous separate strands. Belling's hypothesis (1933) escaped this difficulty by postulating that the genes are connected by extensive non-genic material which produces crossovers in pachytene by shifting of old connections and forming of new ones along the lines of minimum distance at half-twists.

A modification of this hypothesis is possible with chromatids, constituted as suggested above, even if the DNA is continuous, by supposing them to be rather plastic where unprotected by internal synapsis of tandem duplications. The duplicated, physiologically active regions correspond to Belling's genes and the unduplicated, physiologically inert regions to his intergenic material.

Under this hypothesis, corresponding regions of the leptotene chromosomes, already with double amounts of DNA (Swift, 1950a, b) and with physiologically active regions already forming chromomeres, attract each other to give zygotene. At pachytene, each chromosome divides lengthwise by equational apportionment of ultimate strands into sister-chromatids. The results of Taylor, Woods, and Hughes (1957) with radioactively marked chromosomes seem to require, as they note, that all newly synthesized strands be apportioned as a block.

We assume that exchanges between ultimate strands are highly improbable if they are parallel, whether within or between chromatids, but that exchange occurs more or less frequently between the crossed strands of the homologous chromatids that are most closely pressed together at an overlap point, established, as in Belling's hypothesis, by accidents of position and pairing in synapsis. We must suppose, moreover, that if such exchange begins, it continues throughout these two chromatids which thus give rise to shortened crossover chromatids. It appears that the greater the length of an overlap, the greater the chance of exchange at the overlap points (Rendel, 1958). We must also suppose that there is more or less twisting of sister-strands along each chromosome in order to account for the randomness of the pairs of homologous chromatids that exchange in successive chiasmata, but that this does not bring about sister-strand crossing-over (Weinstein, 1936).

The requirements for accounting for the phenomena of crossing-over are undoubtedly somewhat severe if the chromatids contain many strands. Yet it seems necessary to consider the possibilities in view of the situation revealed by the electron microscope.

Continuing with this model, we may suppose that short double exchanges may occur under very rare conditions among groups of parallel strands, leading to unstable mutations of the conversion type if between strands of homologous chromatids of a heterozygote. Mutations might also arise occasionally from such double exchanges between paired strands of differentiated tandem duplications within a coiled chromomere.

Terminology

Within the boundaries established by rearrangements, we expect a hierarchy of structures with varying degrees of physiological complexity and differentiation, culminating perhaps in differences so great that no relationship can be recognized. We need a hierarchy of terms. Locus should I think be used for systems of multiple

alleles, however complex, as long as no cleavage has actually been observed. To divide a locus on the basis of physiological effects alone involves a dangerous begging of the question of structure. If, however, cleavage has been observed, it becomes a question whether one prefers to recognize a single locus with subloci separable at rates like those of mutation, or a superlocus or locus cluster with component loci. In the former case, we should recognize a qualified allelism between separable components. One might use "*euallelic*" for strict allelism as far as known, and thus *aneuallelic* where there is very rare crossing-over within what seems most convenient to recognize as a locus, on the occasions in which it seems necessary to make a distinction. The terms, identical and non-identical alleles, which have been suggested (Demerec, 1956), seem confusing since strict alleles need not be identical physiologically. The terms homoallelic and heteroallelic, which have also been suggested (Roman, 1956), would be appropriate except that they seem too much like the terms homallelic and heterallelic which have been used since 1937 (Wright, 1937) for important concepts in population genetics. A population is homallelic with respect to a locus if all individuals are homozygous in the same sense; otherwise it is heterallelic. An isogenic population is homallelic in all loci.

It would seem well to restrict *pseudoallelic* to description of genetic relations between loci of a recognized superlocus or locus cluster, because of its implication of spuriousness.

Heterochromatin

Selection may operate to build up a large number of undifferentiated longitudinal replications of a physiological gene. The suggestion of Casperssen (1941), developed further by Pontecorvo (1944), that heterochromatin may consist of many replications of a few genes, the products of which are needed in bulk, is an attractive one.

This could account (Hannah, 1951) for the irregular organization, the easy disruption in some but not all cases (in contrast with the postulated stabilization by one tandem duplication), the tendencies toward apparent non-homologous pairing after rearrangement, easy transposition of probable heterochromatin blocks in maize (McClintock, 1951; Brink and Nilan, 1952), relative dispensability, including that of supernumerary chromosomes (Beermann, 1956), restriction of observed differential effects on characters to quantitative modifying action (Mather, 1944; Goldschmidt, Hannah, and Piternick, 1951), and the inhibition of the activity of euchromatic genes brought into proximity (Schultz, 1936).

Large masses of the same active material might be expected to become low points in concentration gradients of raw materials and high points in concentration gradients of products (especially at low temperatures) (Sturtevant, 1925; Offermann, 1935). The V-type position effects of masses of heterochromatin on unadjusted genes (Schultz, 1936), the inhibitory effect on adjacent genes of the transposable elements in maize (McClintock, 1951; Brink and Nilan, 1952), the inhibitory effect of extra Y-chromosomes on sensitive genes of *Drosophila* throughout the genome recently described by Cooper (1956), and its greater inhibitory effect on other heterochromatin and hence on the V-type position effect itself by double negative action (Gowen and Gay, 1934) may be examples. So also depletion of raw materials may account for negative heteropycnosis under some circumstances in contrast with positive under more favorable circumstances, for slow multiplica-

tion where euchromatin is multiplying rapidly (salivary chromosomes of diptera), and perhaps for unequal effects in different cell lineages (variegation).

One might also, perhaps, expect that the accumulation of many tandem replicates of the same genic material would lead to non-equational mitosis in it and hence to variegation for a different reason. Also to be expected is relatively frequent irregularity in meiosis by unequal crossing-over, especially if there are great differences in number of replicates in parental heterochromatic regions, and thus a sort of semi-Mendelian heredity of any effect. This should simulate somewhat the effect of multifactorial heredity and occasionally contribute something to the mode of heredity of quantitative variability.

These are conjectures. I suspect that experimental work of the next few years, perhaps work to be reported here, will do much to clear up the relations between structural and physiological genes and between euchromatic and heterochromatic ones.

Physiological Genetics

The idea that genes control the properties of cells by determining the enzymes that are present goes back in a sense to the earliest years of the century, and the idea that genes act by imposing specific patterns in the process of synthesis of macromolecules whether in their own duplication, in the production of antigenic specificity, or in that of the enzymes is also far from recent (cf. Wright, 1941). These conjectures had, however, little or no real impact on general physiology. Until very recently, the textbooks in that subject rarely made any mention of the gene and treated the cell as the ultimate unit of life.

The real breakthrough came recently when systematic exploration of the control of elementary processes of the mould *Neurospora* was made by the combined efforts of a geneticist and a biochemist, Beadle and Tatum. Now we have a rapid expansion of biochemical genetics or genetic biochemistry (Haldane, 1954; Wagner and Mitchell, 1955). What had been two almost airtight compartments of biology are now inextricably interwoven.

Studies of the relations of gene and cytoplasm and of cytoplasmic heredity began early with Correns and have continued on a relatively modest side. Nevertheless, some of the most important results of the recent period have been obtained in this field. I regret that the time at my disposal forbids any discussion here. The time seems ripe for a major breakthrough.

Developmental Genetics

A step up in the biological hierarchy is the problem of development. There is not much of a non-speculative character that can be said of histogenesis, which rests on the still enigmatic relations of genes and cytoplasm to which we have referred. With respect to morphogenesis there is at least increasing contact between geneticists and embryologists. There was a period not long ago when experimental embryologists would trace the interactions of various factors in the development of an organ—pressures and tensions, inductions, hormones, neural stimulation, environmental conditions—and perhaps, at the end, list heredity as a sort of magic that operated through other than physiological channels. More recently, many trained embryologists have turned to genetically determined abnormalities as useful

material, with illuminating results. The systematic study of the interaction effect of numerous loci on particular characters is especially promising.

The complete analysis of the development of a higher organism nevertheless remains one of the most intractable problems of science. Perhaps it is beyond human grasp. But I suspect that there will be great advances in understanding and am sure that this can come about only in the conjunction of the two disciplines.

Beyond morphogenesis is the genetics of behavior on which a beginning has been made.

Population Genetics

Finally, we pass to the level of the genetics of populations. To a considerable extent this can bypass the levels of developmental and cellular biology and treat populations merely as systems of gene or aberrational frequencies, subject to various abstract pressures. There has been a considerable mathematical development of theory along this line from fairly early in our history. Somewhat divergent conceptions have been reached which I have discussed elsewhere (for example, in Jepsen, Simpson, and Mayr, 1949) and do not propose to go into here. I may say, however, that it is apparent that we need a more adequate theory of quantitative variability, based on generalizations at the levels of physiological and developmental genetics and also more understanding of the implications of population structures and ecologic relations.

It is hopeful that the recent period has been characterized by rapidly increasing interest in the genetics of human populations, in the study of laboratory population of various organisms, and in studies on a large scale of the genetic properties of populations in nature, such as those under the leadership of Dobzhansky and of Clausen.

In this last we come full circle. The geneticists, systematists, field naturalists, and even the paleontologist find that they have problems that can only be solved by joining forces (Jepsen, Simpson, and Mayr, 1949).

Conclusion

A subject which at its origin seemed to many to be destined merely to grind out endless 3:1 ratios has penetrated through the biologic sciences from the level of the individual down to a new biological entity at the level of the macromolecule and back through various disciplines to the level of the population. It has become inextricably linked with cytology, biochemistry, general physiology, experimental embryology, the behavioral sciences, ecology, systematics, and even paleontology. If it should ever disappear as a separate discipline, it will only be because all theoretical biology has been bound together into a single field, to a large extent through its efforts. In relating the formerly mysterious, almost magic, subject of heredity to the chemistry of a particular class of molecules, it has also played a major role in binding all science into one coherent whole.

If this unified science indicates from one point of view that all that pertains to the most complex organisms, including man and his behavior, is merely the resultant of laws of nature governing molecules, atoms, and ultimately elementary physical particles and their fields, it indicates from the opposite point of view (Wright, 1953) that these same molecules, atoms, and particles are little creatures whose mainsprings of action must be essentially similar in kind to those of their derivatives, the complex creatures in which Dr. Wheeler took such delight.

REFERENCES

AVERY, O. T., C. M. MACLEOD, and M. MCCARTY. 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcal types: Induction of transformation by a desoxyribonucleic acid fraction isolated from Pneumococcus Type III. J. Exp. Med. *79*: 137–157.

BARISH, N., and A. S. FOX. 1956. Immunogenetic studies of pseudoallelism in *Drosophila melanogaster*. II. Antigenic effects of the vermilion pseudoalleles. Genetics *41*: 45–57.

BATESON, W. 1909. Mendel's Principles of Heredity. Cambridge: Cambridge Univ. Press.

BEERMAN, W. 1956. Nuclear differentiation and functional morphology of chromosomes. Sympos. Quant. Biol. *21*: 217–232.

BELLING, J. 1928. The ultimate chromomeres of lilium and aloe with regard to the numbers of genes. Univ. California Pub. Bot. *14*: 307–318.

——— 1933. Crossing over and gene rearrangement in flowering plants. Genetics *18*: 388–413.

BENZER, S. 1957. The elementary units of heredity. *In* A Symposium on the Chemical Basis of Heredity, eds., W. D. MCELROY and B. GLASS, pp. 70–93. Baltimore: The Johns Hopkins Press.

BOIVIN, A., R. VENDRELY, and C. VENDRELY. 1948. L'acide désoxyribonucléique du noyau cellulaire, dépositaire des caractères héréditaires: Arguments d'ordre analytique. C. rend. Acad. sc. *226*: 1061–1063.

BONNER, D. M. 1956. The genetic unit. Sympos. Quant. Biol. *21*: 163–170.

BRIDGES, C. B. 1935. Salivary chromosome maps with a key to the banding of the chromosomes of *Drosophila melanogaster*. J. Hered. *26*: 60–64.

BRIDGES, C. B., and K. S. BREHME. 1944. The mutants of *Drosophila melanogaster*. Pub. Carnegie Inst. Washington no. 552.

BRINK, R. A., and R. A. NILAN. 1952. The relation between light variegated and medium variegated pericarp in maize. Genetics *37*: 519–544.

CASPERSSON, T. 1941. Studien über den Eiweissumsatz der Zelle. Naturwissenschaften *29*: 33–43.

CHOVNICK, A., and A. S. FOX. 1953. Immunogenetic studies of pseudoallelism in *Drosophila melanogaster*. I. Antigenic effects of the lozenge pseudoalleles. Proc. Nat. Acad. Sc. U.S. *39*: 1035–1043.

COOPER, K. W. 1956. Phenotypic effects of Y chromosome hyperploidy in *Drosophila melanogaster* and their relation to variegation. Genetics *41*: 242–264.

CUÉNOT, L. 1904. L'hérédité de la pigmentation chez les souris. Arch. zool. exp., Par. Ser. 4. *2*: Notes et Revues xlv–lvi.

DELBRUCK, M., and G. S. STENT. 1957. On the mechanism of DNA replication. *In* A Symposium on the Chemical Basis of Heredity, eds. W. D. MCELROY and B. GLASS, pp. 699–736. Baltimore: The Johns Hopkins Press.

DEMEREC, M. 1928. Mutable characters of *Drosophila virilis*. I. Reddish-alpha body character. Genetics *13*: 359–388.

——— 1956. A comparative study of certain gene loci in *Salmonella*. Sympos. Quant. Biol. *21*: 113–121.

DUBININ, N. P. 1929. Allelomorphentreppen bei *Drosophila melanogaster*. Biol. Zbl. *49*: 328–339.

DUNN, L. C. 1956. Analysis of a complex gene in the house mouse. Sympos. Quant. Biol. *21*: 187–195.

EAST, E. M. 1929. Self-sterility. Bibliogr. genet., Gravenh. *5*: 331–370.

FRIEDRICH-FREKSA, H. 1940. Bei der Chromosomenkonjugation wirksame Kräfte und ihre Bedeutung für die identische Verdopplung von Nucleoproteinen. Naturwissenschaften *28*: 376–379.

GILES, N. H. 1955. Forward and back mutation at specific loci in *Neurospora*. Brookhaven Sympos. Biol. *8*: 103–125.

GOLDSCHMIDT, R. 1946. Position effect and the theory of the corpuscular gene. Experientia, Basel. *2*: 197–203, 250–256.

GOLDSCHMIDT, R. B., A. HANNAH, and L. KELLEN-PITERNICK. 1951. The podoptera effect in *Drosophila melanogaster*. Univ. California Pub. Zool. *55*: 67–293.

GORDON, M. 1947. Speciation in fishes: Distribution in time and space of seven dominant multiple alleles in *Platypoecilus maculatus*. Advance. Genet. *1*: 95–132.

GOWEN, J. W., and E. H. GAY. 1934. Chromosome constitution and behavior in eversporting and mottling in *Drosophila melanogaster*. Genetics *19*: 189–208.

GREEN, M. M., and K. C. GREEN. 1956. A cytogenetic analysis of the lozenge pseudoalleles in *Drosophila*. Zschr. indukt. Abstamm. *87*: 708–721.

HALDANE, J. B. S. 1954. The Biochemistry of Genetics. New York: Macmillan.

HANNAH, A. 1951. Localization and function of heterochromatin in *Drosophila melanogaster*. Advance. Genet. *4*: 87–125.

IRWIN, M. R. 1947. Immunogenetics. Advance. Genet. *1*: 133–159.

JEPSON, G. L., E. MAYR, and G. G. SIMPSON (eds.). 1949. Genetics, Paleontology and Evolution. Princeton: Princeton Univ. Press.

KAUFMANN, B. P., and M. R. MCDONALD. 1956. Organization of the chromosome. Sympos. Quant. Biol. *21*: 233–246.

LAUGHNAN, J. R. 1955. Structural and functional bases for the action of the A alleles in maize. Am. Natur. *89*: 91–103.

LEWIS, D. 1954. Comparative incompatibility in angiosperms and fungi. Advance. Genet. *6*: 235–285.

LEWIS, E. B. 1945. The relation of repeats to position effect in *Drosophila melanogaster*. Genetics *30*: 137–166.

LINDEGREN, C. C. 1953. Gene conversion in saccharomyces. J. Genet., Cambr. *51*: 625–637.

MCCLINTOCK, B. 1951. Chromosome organization and genic expression. Sympos. Quant. Biol. *16*: 13–47.

MATHER, K. 1944. The genetical activity of heterochromatin. Proc. R. Soc. Ser. B. Biol. Sc., Lond. *132*: 308–332.

MAZIA, D. 1954. The particulate organization of the chromosome. Proc. Nat. Acad. Sc. U.S. *40*: 521–527.

MAZIA, D., and W. S. PLAUT. 1955. The distribution of parental material in chromosome reproduction. Biol. Bull. *109*: 335–336. (abstract)

METZ, C. 1947. Duplication of chromosome parts as a factor in evolution. Am. Natur. *81*: 81–103.

METZ, C. W. 1938. Chromosome behavior, inheritance and sex determination in *Sciara*. Am. Natur. *72*: 485–520.

MIRSKY, A. E., and H. RIS. 1949. Variable and constant components of chromosomes. Nature, Lond., *163*: 666–667.

MITCHELL, M. B. 1955. Aberrant recombination of pyridoxine mutants of *Neurospora*. Proc. Nat. Acad. Sc. U.S. *41*: 215–220.

MORGAN, T. H. 1922. On the mechanism of heredity. Proc. R. Soc. Ser. B. Biol. Sc., Lond. *94*: 162–197.

MULLER, H. J. 1929. The gene as the basis of life. Proc. Internat. Congr. Plant Sc. *1*: 897–921.

——— 1955. On the relation between chromosome changes and gene mutations. Brookhaven Sympos. Biol. *8*: 126–147.

NABOURS, R. K. 1930. Mutations and allelomorphism in the grouse locusts. (Tettigidae, orthoptera). Proc. Nat. Acad. Sc. U.S. *16*: 350–353.

OFFERMANN, C. A. 1935. The position effect and its bearing on genetics. Bull. Acad. Sc. U.R.S.S. Cl. Sc. Math. Nat. *1*: 129–152.

OLIVER, C. P. 1940. A reversion to wild-type associated with crossing-over in *Drosophila melanogaster*. Proc. Nat. Acad. Sc. U.S. 26: 452–454.

PAINTER, T. S. 1934. A new method for the study of chromosome aberrations and the plotting of chromosome maps in *Drosophila melanogaster*. Genetics *19*: 175–188.

PATTERSON, J. T.; W. STONE; S. BEDICHEK; and M. SUCHE. 1934. The production of translocations in *Drosophila*. Am. Natur. *68*: 359–369.

PONTECORVO, G. 1944. Structure of heterochromatin. Nature, Lond. *153*: 365–367.

PONTECORVO, G., and J. A. ROPER. 1956. Resolving power of genetic analysis. Nature, Lond. *178*: 83–84.

PRITCHARD, R. H. 1955. The linear arrangement of a series of alleles of *Aspergillus nidulans*. Heredity *9*: 343–371.

RENDEL, J. M. 1958. The effect of age on the relationship between coincidence and crossing over in *Drosophila melanogaster*. Genetics *43*: 207–214.

RIS, H. 1957. Chromosome structure. *In* A Symposium on the Chemical Basis of Heredity, eds. W. D. MCELROY and B. GLASS, pp. 23–69. Baltimore: The Johns Hopkins Press.

ROMAN, H. 1956. Studies of gene mutation in Saccharomyces. Sympos. Quant. Biol. *21*: 175–185.

SCHULTZ, J. 1936. Variegation in *Drosophila* and the inert chromosome regions. Proc. Nat. Acad. Sc. U.S. *22*: 27–33.

SEREBROVSKY, A. S. 1930. Untersuchungen über Treppenallelomorphismus. IV. Transgenation Scute-6 und ein Fall des "Nicht-allelomorphismus" von Gliedern einer Allelomorphenreihe bei *Drosophila melanogaster*. Arch. Entwmech. *122*: 88–104.

STADLER, L. J. 1954. The gene. Science *120*: 811–819.

STORMONT, C. 1955. Linked genes, pseudoalleles and blood groups. Am. Natur. *89*: 105–116.

STREISINGER, G., and N. C. FRANKLIN. 1956. Mutation and recombination at the host range genetic region of Phage T2. Sympos. Quant. Biol. *21*: 103–111.

STURTEVANT, A. H. 1925. The effects of unequal crossing over at the bar locus in *Drosophila*. Genetics *10*: 117–147.

SWIFT, H. 1950. The constancy of desoxyribose nucleic acid in plant nuclei. Proc. Nat. Acad. Sc. U.S. *36*: 643–654.

SWIFT, H. F. 1950. The desoxyribose nucleic acid content of animal nuclei. Physiol. Zool. *23*: 169–198.

TAN, C. C. 1946. Mosaic dominance in the inheritance of color patterns in the lady-bird beetle, *Harmonia axyridis*. Genetics *31*: 195–210.

TANAKA, Y. 1953. Genetics of the silkworm, *Bombyx mori*. Advance. Genet. *5*: 239–317.

TAYLOR, J. H., P. S. WOODS, and W. L. HUGHES. 1957. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. Proc. Nat. Acad. Sc. U.S. *43*: 122–128.

WAGNER, R. F., and H. K. MITCHELL. 1955. Genetics and Metabolism. New York: J. Wiley & Sons.

WATSON, J. D., and F. H. C. CRICK. 1953. The structure of DNA. Sympos. Quant. Biol. *18*: 123–131.

WEINSTEIN, A. 1936. The theory of multiple-strand crossing over. Genetics *21*: 155–199.

WENRICH, D. H. 1916. The spermatogenesis of *Phrynotettix magnus*, with special reference to synapsis and the individuality of the chromosomes. Bull. Mus. Comp. Zool. Harvard *60*: 57–133.

WHEELER, W. M. 1923. The dry-rot of our academic biology. Science *57*: 61–71.

WHITING, P. W. 1943. Multiple alleles in complementary sex determination of *Habrobracon*. Genetics *28*: 365–382.

——— 1954. Comparable mutant eye colors in *Mormoniella* and *Pachycrepoideus* (Hymenoptera: Pteromalidae). Evolution *8*: 135–147.

WIENER, A. S., and I. B. WEXLER. 1952. The mosaic structure of red blood cell agglutinogens. Bact. Rev., Balt. *16*: 69–87.

WINKLER, H. 1930. Die Konversion der Gene. Jena: G. Fischer.

WRIGHT, S. 1916. An intensive study of the inheritance of color and of other coat characters in guinea-pigs, with especial reference to graded variations. Pub. Carnegie Inst. Washington No. *241*: 59–160.

——— 1937. The distribution of gene frequencies in populations. Proc. Nat. Acad. Sc. U.S. *23*: 307–320.

——— 1941. The physiology of the gene. Physiol. Rev. *21*: 487–527.

——— 1949. Estimates of amounts of melanin in the hair of diverse genotypes of the guinea pig, from transformation of empirical grades. Genetics *34*: 245–271.

——— 1953. Gene and organism. Am. Natur. *87*: 5–18.

PANEL DISCUSSIONS

1. Panel on the Teaching of Genetics. Reported by BENTLEY GLASS, Moderator
2. A Discussion on *Drosophila* Terminology. Reported by K. BREHME-WARREN and C. P. OLIVER

PANEL ON THE TEACHING OF GENETICS

Reported by Bentley Glass, Moderator

A PANEL DISCUSSION on the teaching of genetics was held on August 23, as a direct response to a resolution of the General Assembly of the International Union of Biological Sciences held in Rome in 1955, to the effect that far too little attention is given in international biological congresses to problems of teaching. The panel members were Professors Curt Stern (University of California), William K. Baker (University of Chicago), and Herschel Roman (University of Washington). Bentley Glass (Johns Hopkins University) served as Moderator.

Professor Stern addressed himself to the major question of the proper presentation of the subject of genetics to a variety of students differing in age, ability, and aims. He disclaimed the view that any one pattern of teaching could fit all situations, but, speaking on the basis of his personal experience in teaching large classes of students at Berkeley, very few of whom become professional geneticists, he expressed the feeling that genetics should be taught, first and foremost, as a part of general biology, that is to say, as contributing to a general understanding of organisms. Second, it should be taught as a scientific discipline, an introduction to scientific thinking in general. How elementary observations lead to the establishment of central concepts, how general concepts become modified through the decades by alteration and substitution of ideas, how different disciplines originally quite independent become fused—these are among the most valuable outcomes of education in such a science as genetics. Professor Stern also emphasized the great importance of visual aids, and in particular the superiority of demonstrations of actual materials and the use of charts over the all-too-rapid exposure to lantern slides in darkness that prevents note-taking and conduces to sleep.

Professor Baker's remarks focused on the qualities of the great teacher who inspires students to become critically minded as well as enthusiastic scientists. One of his own teachers, Warren P. Spencer, a man who has inspired many able persons to enter the field of genetics, was a stimulating lecturer, who raised many broad questions relating to evolution and posed many stimulating special problems in the laboratory. Other teachers, great in their own way, were not necessarily good lecturers. Professor Baker mentioned one, molded in the tradition of Agassiz and Brooks, who used to give his student a chair, a microscope, and some *Drosophila* cultures, and say: Now find yourself a problem! But he was always interested in his student's progress and always willing to talk at length about the problem. Another teacher was great primarily because he could always talk about the new ideas developing in the field of genetics and conveyed a feeling of the dynamic development of experimental science. All these teachers possessed in common one quality: they had a firm grasp of genetics—its principles, its problems, and a vast store of detailed knowledge about the genetics of one or more species. In this respect it should be emphasized, Baker thought, that the vast mutual interpenetration of genetics and other subjects, such as cytology, biochemistry, and the study of development, has not at all reduced the need for the acquisition by the student

of a thorough grounding in basic genetic principles. If anything, it has increased that need.

Professor Roman continued the consideration of the role of the teacher, with special emphasis on the recruitment of new geneticists. There have been notable examples in genetics of the growth of an outstanding group of students around a single great pioneer. Could one, Professor Roman wondered, make a scientific reputation by surrounding himself with able graduate students to make the reputation for him? Thinking of the brilliant group who surrounded R. A. Emerson at Cornell in the 1920's and early 1930's he wondered what had been the special ingredient. Was it Emerson's own endless patience and devotion to his students, and his self-effacement in the initial phases of their public careers? Checking farther, he found that a number of members of this group as undergraduates had received an impetus towards biology, and towards genetics in particular, from a professor who had taught them at the University of Nebraska and steered them to Emerson at Cornell. A man of no wide reputation himself, his contributions to genetics through his recruitment of some of our ablest colleagues possibly far outweighs in ultimate significance the stacks of papers in which we take such ephemeral pride! What is the secret of such success in recruitment? Surely it is compounded of many things: the ability to recognize in an immature student the gifts of future greatness; the personal interest that early reveals to the student the humanity of science and the scientist; the devotion to truth that inspires emulation; the patience that helps a student to realize he too may one day become a real scientist; and the encouragement when personal problems are difficult. One ought not to underestimate the importance of a small, paid job at the right time. Yet in all of this, no coddling. The student must learn to stand on his own feet.

Professor Roman also directed attention to the changes in the content of courses which ought to follow the development of new areas of genetics. It is extraordinary, and not creditable, that many laboratory programs in elementary genetics are still limited to counts of corn ratios, a few crosses of *Drosophila,* and analysis of some human pedigrees—in other words, with the genetics that existed in 1935. Considering that the science of genetics has developed entirely since 1900, the date of the Mendelian rediscoveries, it seems incredible that 40 per cent of its period of growth is virtually unrepresented in what we teach in the laboratory. It is by no means impossible to teach microbial genetics, biochemical genetics, or population genetics in the elementary laboratory if thought and care are given to the planning. To take microbial genetics as an example, yeast strains suitable for laboratory problems, such as tests for allelism, can be sent anywhere very easily (by immersing filter paper in yeast suspension, drying, covering with aluminum foil, and mailing in an ordinary envelope). Although some expense is involved in equipping the laboratory with pipettes and glassware, it is not greater than the expense of elementary bacteriology, which likewise requires sterile techniques. The medium is easier to prepare and less expensive than many bacteriological media. Dramatic experiments can be done with phage, *E. coli, Chlamydomonas,* and higher organisms such as *Paramecium.*

A third teaching problem touched on by Professor Roman was that of the requirements for training in genetics. He suggested the following: mathematics through calculus, statistics, physical chemistry, biochemistry, microbiology, and human or medical genetics. Especially important, within the area of genetics itself, is diversity of training with respect to a variety of organisms. The worker who has personal experience with the genetics of a micro-organism, a plant such as maize,

and an animal such as *Drosophila,* is far better prepared to attack a variety of problems and to teach with a comprehensive viewpoint than is a person whose preparation has been restricted to work on a single organism. At least one speaker, in the later discussion, deplored the compartmentalization of genetics.

In the general discussion which followed the remarks of the three panel members, a number of significant questions were raised although the occasion did not permit any of them to be explored very fully. It seemed to be a consensus that prolonged and systematic study should be given to the consideration of these problems.

One question raised was whether non-laboratory genetics courses were adequate for certain groups of students, especially those not intending to major in biology. One speaker deplored the tendency to substitute demonstrations for individual laboratory experience. A related question concerned the employment of visual aids. Should lectures by notable geneticists be filmed and employed in place of presentation by the local teacher? Can films be developed that will make the process of learning laboratory techniques easier? On the other side, can laboratory space and equipment be provided for all students who may want to take at least an elementary course in genetics or who ought to do so? Can a handbook of laboratory methods and problems be compiled to modernize our laboratory teaching? For micro-organisms and other suitable species, can services be developed comparable to the Drosophila Information Service, together with stock centres which supply teachers with suitable genetic stocks for laboratory work? Baker pointed out that students who can successfully solve the standard types of genetics problems given in textbooks are often unable to analyze their own data in a parallel way. Is there any more effective way of training students to analyze data and to draw valid conclusions in laboratory situations? A plea was made by another speaker for more emphasis on the variability existing within populations.

In discussing the classroom approach, several speakers reiterated Stern's opinion that the historical approach is of paramount value. Ludwig stated that it had been grossly neglected in Germany. Stebbins emphasized that a part of its value is to explain why some scientists (for example, Mendel, Muller) succeed where so many predecessors failed, and to enable the student to distinguish good scientific directions from fruitless ones.

The rising interest in medical genetics raises several questions. Should the study of human genetics be focused on general genetic principles, or on problems of social and clinical importance? Should the medical school course be one in human genetics or in general genetics? Norma Ford Walker recommended the former, inasmuch as in Canada general genetics is taught in the high schools. At Johns Hopkins, on the other hand, the new required course in the medical curriculum will be general in nature, and specialized clinical interests will be developed in elective advanced courses and seminars; but then, in the United States many students have had almost no acquaintance with genetics prior to entering medical school. Stern emphasized that human genetics has largely grown out of the work on fruitflies and peas, and that a general basis is always the soundest foundation for specialized work. A particular aspect which called for animated discussion was the treatment to be given to questions of genetic differences between human races, and to the problem of racial discrimination. Stern emphasized that the principle to be stressed is that genetic differences in frequencies of alleles exist within racial groups as well as between them. In his own classes he dramatizes this fact by distributing PTC paper to the students, asking them to taste it, and then having them report by racial groups (whites, Orientals). The data then make it clear

that the same difference between tasters and non-tasters exists in both racial groups, although the frequencies of non-tasters are different. Thus students are encouraged to approach such problems scientifically instead of emotionally. Another speaker pointed out that it is desirable to familiarize students in areas of mixed human population as early as possible with the backcross ratio (1:1) as well as the F_2 ratio.

There was expression of the need for students of genetics to have increased opportunities to acquaint themselves with genetic materials. Perhaps genetics needs a Woods Hole laboratory. (Some recently established summer laboratories in the United States may indeed become an equivalent.) Professor Müntzing described the opportunities for summer work by students in Sweden. After a formal course running from January to May, a student may enroll in a fourteen-day summer course in which he sees many types of living material—plant, animal, human—available in other institutions in the same province. He comes into contact with demonstrators and research workers actively working on genetic problems, and develops a first-hand acquaintance with the meaning and significance of clones, pure lines, populations, mutant forms, etc., leading to a real comprehension of genetic variability.

Finally, there was considerable discussion of the need for teachers at the college and university level to pay more heed to the problems of teaching genetics, as a part of biology, at the secondary, and indeed at the elementary, school levels. The relations between working geneticists and high school and elementary school teachers of biology should be greatly improved. Very often the real bases of a decision to enter a career in science are formed before the college years begin, and elementary school and high school teachers are consequently most influential. In the United States the Summer Science Institutes for high school teachers, supported by grants from the National Science Foundation and other governmental agencies, are beginning to produce results in this area. Testimony to their generally high value was given, and geneticists from Canada, New Zealand, and other countries were heard to express a wish that something of the same nature could be begun in their own lands. Professor Roman spoke of the fine response observed in his own institution when members of the Botany Department extended an invitation to high school teachers to come once a week for a mutual conference on aims and methods, including the demonstration of simple experiments suitable for their own teaching.

It is to be hoped that in the future much more systematic and continuous attention will be given by geneticists to the problems of teaching and recruitment in their field. If that happens, the next panel discussion of the subject might be more than exploratory in character.

A DISCUSSION ON *DROSOPHILA* TERMINOLOGY

Reported by K. Brehme-Warren and C. P. Oliver

A MEETING to discuss problems of *Drosophila* terminology was held on August 21, 1958, with C. P. Oliver presiding. The first topic under discussion was that of general mutant symbols; in certain allelic series, where both dominant and recessive alleles occur, the designations now used do not necessarily express their dominance relations. For example, eyeless-dominant is symbolized as ey^{D}, while the two recessives, eyeless-Russian and eyeless-Novitski are respectively ey^{R} and ey^{Nov}. It was the consensus of opinion of the meeting that, in the future, recessive alleles be given a lower case superscript symbol (for example ey^{ru} and ey^{nov} for the above recessives).

The terminology of pseudo-allelic series was discussed at length. E. A. Carlson presented a plan for expressing both locus and phenotype in one term (for example, lozenge-Bar of Stone, which is at the spectacle locus within the lozenge series, would become lz^{speBS}, or when referred to specifically in a publication, would be $'spe^{BS}$). B. Glass reminded the meeting that more than three letters in a superscript are expensive to print, while a superscript to a superscript is both unwieldy to type and prohibitively expensive to print. E. B. Lewis was of the opinion that the apostrophe is not flexible enough to be of general use for *Drosophila* and other organisms where pseudo-allelic series are found. A. S. Fox pointed out that there are two problems to be considered in pseudo-allelic series, that of designating locus and that of designating phenotype. It was felt by J. C. Mossige and C. P. Oliver that a symbol is intended for identification and not for description of phenotype. The general opinion of the meeting seemed to be that expressed by A. H. Sturtevant: the time is not yet ripe for standardized pseudo-allele symbols, and for the present such symbols should be used as are convenient.

Designation of the position of the centromere was next discussed. D. L. Lindsley mentioned the present confusion resulting from the use of the raised dot (chem point in printing terms) for centromere, break point, and attachment point. It was his opinion that the raised dot should be reserved for centromere, slash (diagonal, shill, in printing terms) for break point, and semicolon for attachment. E. B. Lewis agreed that Bridges had set aside the slash for break point. With respect to the raised dot, A. H. Sturtevant pointed out that this kind of symbol can be used in two ways without confusion. No conclusion was reached by the meeting.

The symbolism for attached-X was brought up for discussion by D. L. Lindsley, who finds confusing the use of $\underline{XX}$ and $.=$ for attached-X and $:=$ for doubly attached-X; he suggested using the terms RA (reversed acrocentric), TA (tandem acrocentric), RM (reversed metacentric), and TM (tandem metacentric). Others reminded the meeting that T stands for translocation and R for rearrangement; the gene order, whether literal or reversed, would also be ambiguous.

Objections were raised to the short-hand designations of marker and tester stocks, such as Binscy, whose genotypes may in some cases be found only in DIS.

A. H. Sturtevant pointed out that DIS is not always available. It was the feeling of the meeting that such short-hand terms should not be introduced in the future; or if they are, the author should give in the introduction the genotype represented by the designation.

No attempt was made to formulate a standard terminology. It was the consensus of the meeting that for the present an author should use symbols that are convenient, have the minimum complexity, and are understandable to the reader.

MEMBERS OF CONGRESS

Active Members are listed, with Associate Members marked with the symbol *, non-participants with the symbol †, and children in parentheses.

Aalders, L. E., Dr.—Dominion Experimental Farm, Kentville, N.S., Canada
Aalders, L. E., Mrs.*—Dominion Experimental Farm, Kentville, N.S., Canada
Abbott, U. K., Dr.—Poultry Husbandry Dept., University of California, Davis, Calif., U.S.A.
Abrahamson, S., Dr.—Dept. of Biology, Rutgers University, Newark, N.J., U.S.A.
Adati, S., Prof.—Faculty of Agriculture, Mie University, Tu, Mie-Ken, Japan
Adelberg, E. A., Dr.—University of California, Berkeley 4, Calif., U.S.A.
Adler, R. F., Dr.—7257 S. Jeffery Ave., Chicago, Ill., U.S.A.
Adler, R. F., Mrs.*—7257 S. Jeffery Ave., Chicago, Ill., U.S.A. (Thomas & Robert)
Ahuja, Y. R., Mr.—Dept. of Genetics, University of California, Berkeley 4, Calif., U.S.A.
Alava, A., Dr.†—University of California, Berkeley, Calif., U.S.A.
Alexander, M. L. Dr.—M. D. Anderson Hospital, University of Texas, Houston, Texas, U.S.A.
Allard, R. W., Prof.—College of Agriculture, University of California, Davis, Calif., U.S.A.
Allen, C. P., Mr.—Hyline Research Laboratories, Dalks Center, Iowa, U.S.A.
Allen, F. H., Jr., Dr.—332 Longwood Ave., Boston 15, Mass., U.S.A.
Allen, F. H., Mrs.*—332 Longwood Ave., Boston 15, Mass., U.S.A.
Allen, G., Dr.—National Institutes of Health, Public Health Service, Bethesda, Md., U.S.A.
Allison, A. C., Dr.—Institute for Medical Research, Mill Hill, London, England
Alper, T., Miss—Medical Research Council, Hammersmith Hospital, Ducane Road, London W.12, England
Alspach, B. W., Dr.—Medical School, University of Miami, Miami, Fla., U.S.A.
Alspach, B. W., Mrs.*—Medical School, University of Miami, Miami, Fla., U.S.A. (Adair & Geoffrey)
Altenburg, E., Dr.†—Rice Institute, Houston, Texas, U.S.A.
Altenburg, L. S., Prof.—Rice Institute, Houston, Texas, U.S.A.
Anders, F., Dr.—Zoological Institute, University of Saarlandes, Saarbrücken, Germany
Anderson, R. G., Dr.—Cereal Breeding Laboratory, Box 322, University of Manitoba, Winnipeg, Man., Canada
Anderson, V. E., Dr.—Dight Institute, University of Minnesota, St. Paul 13, Minn., U.S.A.
Anderson, V. E., Mrs.*—1554 N. Pascal St., St. Paul 13, Minn., U.S.A. (Catherine, Carl & Christine)
Anderson, R. E., Dr.—Cornell University, Ithaca, N.Y., U.S.A.
Andrews, J. E., Dr.—Experimental Farm, Lethbridge, Alta., Canada
Angel, J. L., Dr.—Daniel Baugh Institute, Jefferson Medical College, Philadelphia 7, Pa., U.S.A.
Angel, J. L., Mrs.*—307 South 11th St., Philadelphia 7, Pa., U.S.A. (Elizabeth, Stephen & Jonathan)
Annan, M., Prof.—Wagner College, Staten Island, N.Y., U.S.A.
Anstey, T. H., Dr.—Experimental Farm, Summerland, B.C., Canada
Appleyard, R. K., Dr.—United Nations, New York, N.Y., U.S.A.
Arisumi, T., Dr.—U.S.D.A., Columbus, Ohio, U.S.A.
Armstrong, J. M., Dr.—Forage Division, Central Experimental Farm, Ottawa, Ont., Canada
Arnason, T. J., Prof.—Biology Dept., University of Saskatchewan, Saskatoon, Sask., Canada
Arnason, T. J., Mrs.*—Biology Dept., University of Saskatchewan, Saskatoon, Sask., Canada (Steffie & Averil)
Arnason, L., Miss—Biology Dept., University of Saskatchewan, Saskatoon, Sask., Canada
Aruga, H., Dr. —Laboratory of Sericulture, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo, Japan
Auerbach, C., Dr.—Institute of Animal Genetics, Edinburgh, Scotland
Axelrad, A. A., Dr.—Ontario Cancer Institute, University of Toronto, Toronto, Ont., Canada
Axtell, J. D., Dr.—University of Minnesota, St. Paul, Minn., U.S.A.

Bailey, C., Dr.—Rutgers University, New Brunswick, N.J., U.S.A.
Bailey, D., Dr.—Dept. of Zoology, University of Kansas, Lawrence, Kansas, U.S.A.
Bajer, A., Dr.—Plant Physiology Lab., Cracow, Poland
Baker, E., Mr.—Iowa State College, Ames, Iowa, U.S.A.
Baker, H. G., Prof.—University of California, Berkeley 4, Calif., U.S.A.
Baker, L., Mr.—University of Minnesota, St. Paul, Minn., U.S.A.
Baker, W. K., Prof.—Dept. of Zoology, University of Chicago, Chicago, Ill., U.S.A.
Baker, W. K., Mrs.*—Dept. of Zoology, University of Chicago, Chicago, Ill., U.S.A. (Bruce, Ann & Brian)
Bakshi, J. S., Mr.—Botany Dept., Oklahoma State University, Okla., U.S.A.
Balbinder, E., Dr.—Dept. of Genetics, Carnegie Institute, Cold Spring Harbor, N.Y., U.S.A.
Balbinder, E., Mrs.*—Dept. of Genetics, Carnegie Institute, Cold Spring Harbor, N.Y., U.S.A.
Barbacki, S., Dr.—Polish Academy of Sciences, Mazowiecka 53, Poznan, Poland
Barber, H. N., Prof.—University of Tasmania, Hobart, Tasmania, Australia
Barigozzi, C., Prof.—University of Milan, via Celoria 10, Milan, Italy
Barigozzi, C., Mrs.*—University of Milan, via Celoria 10, Milan, Italy
Barr, W. K., Dr.—Dept. of Agriculture, 1439 Randall Ave., Ottawa, Ont., Canada
Barratt, R. W., Prof.—Dartmouth College, Hanover, N.H., U.S.A.
Bartsch, K. H., Dr.—Institut für Tierzuchtforschung, Clausberg/Eisenach, Germany DDR
Basrur, P., Mrs.*—Dept. of Botany, University of Toronto, Toronto, Ont., Canada
Bateman, A. J., Dr.—Christie Hospital & Holt Radium Institute, Wilmslow Rd., Manchester, England
Bateman, N., Dr.—A. R. C. Animal Breeding Research Organization, Dryden Mains, Roslin, Midlothian, Scotland
Bat-Miriam, M., Miss—Israeli Institute for Biological Research, Ness-Ziona, Israel
Battaglia, B., Dr.—Dept. of Zoology, The University, Padua, Italy
Battaglia, E., Dr.—Istituto Botanica, Facolta di Farmacia, Trieste, Italy
Bayley, N.D., Dr.—Dairy Cattle Research Branch, Animal Husbandry Research Division, U.S.D.A., Beltsville, Md., U.S.A.
Bayley, S. T., Dr.—Division of Applied Biology, National Research Council of Canada, 100 Sussex Drive, Ottawa 2, Ont., Canada
Beadle, G. W., Prof.†—Division of Biology, California Institute of Technology, Pasadena, Calif., U.S.A.
Beal, V. C., Mr.—Dairy Dept., Purdue University, Lafayette, Ind., U.S.A.
Beamish, K. I., Dr.—University of British Columbia, Vancouver, B.C., Canada
Beardmore, J. A., Dr.—University of Sheffield, Sheffield, England
Bearn, A. G., Dr.—Rockefeller Institute, 66th St. & York Ave., New York, N.Y., U.S.A.
Bearn, A. G., Mrs.*†—Rockefeller Institute, 66th St. & York Ave., New York, N.Y., U.S.A.
Beasley, A. B., Dr.—Nevis Biological Station, Columbia University, Irvington, N.Y., U.S.A.
Beatty, A. V., Prof.—Biology Dept., Emory University, Atlanta, Ga., U.S.A.
Beatty, R. A., Dr.—A.R.C. Unit of Animal Genetics, West Mains Rd., Edinburgh 9, Scotland
Beaudry, J. R., Prof.—Botanical Institute, University of Montreal, 4101 Sherbrooke St. E., Montreal, P.Q., Canada
Beck, S., Mr.—Dept. of Biology, Brown University, Providence, R.I., U.S.A.
Becker, H. J., Dr.—Dept. of Zoology, University of California, Berkeley, Calif., U.S.A.
Becker, W., Dr.—Experiment Station, State College of Washington, Puyallup, Washington, U.S.A.
Beckstrom, J. H., Miss*—University of Wisconsin, Madison, Wisc., U.S.A.
Behnke, J. A., Mr.—Ronald Press Co., 15 E. 26th St., New York, N.Y., U.S.A.
Bell, A. E., Dr.—Poultry Science Dept., Purdue University, Lafayette, Ind., U.S.A.
Bell, A. E., Mrs.*—Poultry Science Dept., Purdue University, Lafayette, Ind., U.S.A. (Robert, Donald & Diane)
Benazet, J., Mr.*—Room 604, 105 McGill St., Montreal, P.Q., Canada
Benazzi, M., Prof.—Instituto di Zoologia, Universita, Pisa, Italy
Bender, H. A., Dr.—Cresap Laboratory, Northwestern University, Evanston, Ill., U.S.A.
Bender, H. A., Mrs.*—Cresap Laboratory, Northwestern University, Evanston, Ill., U.S.A.
Bender, M. A., Dr.—The Johns Hopkins University, Baltimore, Md., U.S.A.
Bennett, C. J., Dr.—Biology Dept., Northern Illinois University, DeKalb, Ill., U.S.A.
Bennett, C. J., Mrs.*—Biology Dept., Northern Illinois University, DeKalb, Ill., U.S.A.
Bennett, J. H., Prof.—University of Adelaide, Adelaide, South Australia

Benzer, S., Prof.—Biophysical Laboratory, Purdue University, Lafayette, Indiana, U.S.A.
Berg, C., Miss*—Dept. of Plant Breeding, Cornell University, Ithaca, N.Y., U.S.A.
Berg, R. T., Dr.—University of Alberta, Edmonton, Alta., Canada
Berg, R. T., Mrs.*—6826 112 A St., Edmonton, Alta., Canada
Bergbusch, V., Miss—University of Saskatchewán, Saskatoon, Sask., Canada
Bergquist, A., Mrs.—University of Texas, Austin, Texas, U.S.A.
Bernard, C., Dr.—Experimental Farm, Lennoxville, P.Q., Canada
Bernstein, S. E., Dr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Bernström, P., Dr.—Hilleshög Sugar Beet Breeding Institute, Landskrona, Sweden
Bhatnager, P., Dr.—University of Illinois, Urbana, Ill., U.S.A.
Bianchi, A., Dr.—Centro di Genetica del C.N.R., P. Botta, Pavia, Italy
Bianco, A. C., Dr.*—Institute of Microbiology, Rutgers University, New Brunswick, N.J., U.S.A.
Bingefors, S., Dr.—Swedish Seed Association, Uppsala 1, Sweden
Bishop, C. J., Dr.—Canada Department of Agriculture, Experimental Farm, Kentville, N.S., Canada
Bistis, G., Dr.—Biological Laboratory, Harvard University, Cambridge, Mass., U.S.A.
Bistis, G., Mrs.*—Biological Laboratory, Harvard University, Cambridge, Mass., U.S.A.
Black, W. F., Dr.—Sir George Williams College, Drummond St., Montreal, P.Q., Canada
Blackwood, M., Dr.—Botany School, University of Melbourne, Carlton 3, Victoria, Australia
Blain, A., Dr.—Jardin Botanique de Montréal, 4101 Sherbrooke St. E., Montreal, P.Q., Canada
Blanco-Gonzalez, J. L., Dr.—Mision Biologica de Galicia, Pontevedra, Spain
Blanco-Gonzalez, M., Mr.—Mision Biologica de Galicia, Pontevedra, Spain
Blount, J., Dr.—Mount Union College, Alliance, Ohio, U.S.A.
Blumel, J., Dr.—University of Texas Medical Branch, Galveston, Texas, U.S.A.
Bogart, R., Prof.—Oregon State College, Corvallis, Ore., U.S.A.
Bohidar, N., Dr.—Dept. of Statistics, Iowa State College, Ames, Iowa, U.S.A.
Böhme, H., Dr.—Institute for Research of Cultivated Plants, Gatersleben, Kr., Germany DDR.
Bohren, B. B., Dr.—Purdue University, Lafayette, Ind., U.S.A.
Bompiani, G. S., Dr.—presso Minelli, via Borgoguona 38, Rome, Italy
Bonner, D. M., Prof.†—Dept. of Microbiology, Yale University, New Haven, Conn., U.S.A.
Bonvicini, M., Dr.—Istituto di allevamento vegetale per la cerealicoltura, via di Corticella No. 133, Bologna, Italy.
Böök, J. A., Dr.—State Institute for Human Genetics, V Agaten 24, Uppsala, Sweden
Boothroyd, E. R., Dr.—Dept. of Genetics, McGill University, Montreal 2, P.Q., Canada
Borojevic, S., Dr.—Faculty of Agriculture, Novi Sad, Yugoslavia
Bose, S., Dr.—Blandy Experimental Farm, Boyce, Va., U.S.A.
Bostian, C. H., Dr.†—1903 Hillsboro St., Raleigh, N.C., U.S.A.
Bostian, C. H., Mrs. *†—1903 Hillsboro St., Raleigh, N.C., U.S.A.
Bowden, W. M., Dr.—Canada Dept. of Agriculture, Science Service, Ottawa, Ont., Canada
Bowers, C. G., Dr.—Cornell University, Ithaca, N.Y., U.S.A.
Bowman, B., Miss—3804 Towerview Court, Austin, Texas, U.S.A.
Boyd, S., Miss*—Smithsonian Observatory, Boston, Mass., U.S.A.
Boyd, W. C., Prof.—Boston University, Boston, Mass., U.S.A.
Boyd, L. Mrs.*—24 Edward St., Belmont, Mass, U.S.A.
Boyer, S., Dr.—Dept. of Medicine, The Johns Hopkins Hospital, Baltimore, Md., U.S.A.
Boyes, J. W., Prof.—Dept. of Genetics, McGill University, Montreal 2, P.Q., Canada
Boyes, J. W., Mrs.*—484 Strathcona Ave., Montreal, P.Q., Canada (Philip, Barbara, Alan & Margie)
Braver, G., Mr.—Dept. of Zoology & Radiation Laboratory, University of California, Berkeley, Calif., U.S.A.
Brawn, R. I., Prof.—Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Bray, D. F., Mr.—Purdue University, Lafayette, Ind., U.S.A.
Brehme-Warren, K., Dr.—Long Island Biological Laboratory, Cold Spring Harbor, N.Y., U.S.A.
Bresler, J., Dr.—Dept. of Biology, Brown University, Providence, R.I., U.S.A.
Brewbaker, H. D., Dr.—The Great Western Sugar Co., Experiment Station, Longmont, Colo., U.S.A.
Brewbaker, H. D., Mrs.*—P.O. Box 539, Longmont, Colo., U.S.A.
Brewbaker, J. L., Dr.—Brookhaven National Lab., Upton, N.Y., U.S.A.
Brewbaker, J. L., Mrs.*—Middle Island Rd., Yaphank, N.Y., U.S.A. (Paul & Philip)

Brieger, F. G., Dr.—Dept. of Genetics, Escola Sup. Agr., University of Sao Paulo, Piracicaba, Brazil
Brieger, F. G., Mrs.*—Dept. of Genetics, Escola Sup. Agr., University of Sao Paulo, Piracicaba, Brazil
Briles, C. O., Dr.—Arbor Acres Farm Inc., Glastonbury, Conn., U.S.A.
Briles, W. E., Dr.—DeKalb Agricultural Assoc., Blood Typing Lab., DeKalb, Ill., U.S.A.
Brink, M., Miss*†—Dept. of Genetics, University of Wisconsin, Madison Wis., U.S.A.
Brink, R. A., Prof.—Dept. of Genetics, University of Wisconsin, Madison, Wis., U.S.A.
Brink, R. A., Mrs.*†—Dept. of Genetics, University of Wisconsin, Madison, Wis., U.S.A.
Brink, R. F., Mrs.*—4135 W. 16th Ave., Vancouver, B.C., Canada
Brink, V. C., Dr.—University of British Columbia, Vancouver, B.C., Canada
Brito da Cunha, A., Prof.—Universidade de Sao Paulo, Faculdade de Filosofia, Sao Paulo, Brazil
Britton, D. M., Dr.†—Dept. of Botany, Ontario Agricultural College, Guelph, Ont., Canada
Brncic, D., Prof.—Escuela de Medicina, Universidad de Chile, Zanartu 1042, Santiago, Chile
Brooks, J. S., Prof.—Agronomy Dept., Oklahoma State University, Stillwater, Okla., U.S.A. (Kathryn, James, Mary & Michael)
Brooks, M. H., Dr.—Agronomy Dept., Oklahoma State University, Stillwater, Okla., U.S.A.
Brosseau, G. E., Jr., Dr.—Biology Division, Oakridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Brosseau, G. E., Jr., Mrs.*—118 E. Arrowood Rd., Oak Ridge, Tenn., U.S.A. (Carl & Catherine)
Brown, D. F., Mr.*†—Genetics Dept., University of Wisconsin, Madison, Wis., U.S.A.
Brown, H. B., Prof.†—
Brown, J. A. M., Mr.—Purdue University, Lafayette, Indiana, U.S.A.
Brown, M. S., Prof.—Texas Agricultural Experiment Station, College Station, Texas, U.S.A.
Brown, W. L., Dr.—Dept. of Plant Breeding, Pioneer Hybrid Corn Co., Johnston, Iowa, U.S.A.
Bruck, D. L., Mr.—North Carolina State College, Raleigh, N.C., U.S.A.
Bryan, J. H. D., Dr.—318 Curtiss Hall, Iowa State College, Ames, Iowa, U.S.A.
Bryan, J. H. D., Mrs.*—318 Curtiss Hall, Iowa State College, Ames, Iowa, U.S.A. (Mary)
Bubar, J. S., Dr.—Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Buchert, J., Dr.—Connecticut Agricultural Experiment Station, New Haven, Conn., U.S.A.
Buckley, G. F. H., Dr.—Canadian Dept. of Agriculture, Experimental Farm, Harrow, Ont., Canada
Buettner-Janusch, J., Dr.—Laboratory of Physical Anthropology, University of Michigan, Ann Arbor, Mich., U.S.A.
Bull, A. L., Dr.—Wellesley College, Wellesley, Mass., U.S.A.
Bullen, M., Mr.—P.O. Box 31, Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Burdick, A. B., Prof.—Dept. of Biological Sciences, Purdue University, Lafayette, Indiana, U.S.A.
Burnett, J., Dr.—Dept. of Zoology, Michigan State University, East Lansing, Mich., U.S.A.
Burnett, Mr.*—702 Cherry Lane, East Lansing, Mich., U.S.A.
Burnham, C. R., Prof.—Dept. of Agronomy & Plant Genetics, University of Minnesota, St. Paul, Minn., U.S.A.
Burnham, C. R., Mrs.*—Dept. of Agronomy & Plant Genetics, University of Minnesota, St. Paul, Minn., U.S.A. (Barbara)
Burnham, S., Miss*—Dept. of Agronomy & Plant Genetics, University of Minnesota, St. Paul, Minn., U.S.A.
Burris, M. J., Mr.—7101 Highland Ave., Springfield, Va., U.S.A.
Burton, G. W., Dr.—Georgia Coastal Plain Experiment Station, Tifton, Ga., U.S.A.
Buss, E., Dr.—Pennsylvania State University, State College, Pa., U.S.A.
Butler, L., Prof.—Dept. of Zoology, University of Toronto, Toronto, Ont., Canada
Butler, L., Mrs.*—103 Douglas Ave., Oakville, Ont., Canada (Linda)
Butzel, H. M., Jr., Dr.—Biology Dept., Union College, Schenectady, N.Y., U.S.A.
Bywaters, J. H., Dr.—The William H. Miner Agriculture Research Institute, Chazy, N.Y., U.S.A.
Bywaters, J. H., Mrs.*—The William H. Miner Agriculture Research Institute, Chazy, N.Y., U.S.A.

Calef, E., Dr.—Istituto di Genetica, via S. Epifanio 14, Pavia, Italy
Callaghan, J., Mr.—Botany Dept., Pennsylvania State University, University Park, Pa., U.S.A.
Callen, E. O., Dr.*—Box 202, Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Camara, A., Prof.—Estacao Agronomica Nacional, Sacavem, Portugal
Cameron, D. R., Dr.—Dept. of Genetics, University of California, Berkeley 4, Calif., U.S.A.
Cameron, J., Dr.—Dept. of Horticulture, University of California, Riverside, Calif., U.S.A.
Caminita, B. H., Mrs.—U.S. Office of Naval Research, Washington 25, D.C., U.S.A. (Ludwig & Richard)
Campbell, I. M., Dr.—Forest Insect Laboratory, Sault Ste. Marie, Ont., Canada
Campbell, I. M., Mrs.—Forest Insect Laboratory, Sault Ste. Marie, Ont., Canada
Carlson, E., Dr.—Biology Dept., Queen's University, Kingston, Ont., Canada
Carman, G. M., Dr.—Central Experimental Farm, Ottawa, Ont., Canada
Carpi, M. D. A., Dr.†—Instituto Español de Moneda Extranjere, Madrid, Spain
Carson, G. L., Dr.—Dept. of Zoology, University of California, Berkeley 4, Calif., U.S.A.
Carson, H. L., Prof.—Dept. of Zoology, Washington University, St. Louis 5, Mo., U.S.A.
Carson, H. L., Mrs.*—Dept. of Zoology, Washington University, St. Louis 5, Mo., U.S.A.
Carter, R. C., Prof.—Virginia Polytechnic Institute, Blacksburg, Va., U.S.A.
Carter, R. C., Mrs.*—405 Hemlock Dr., Blacksburg, Va., U.S.A.
Carver, J. E., Jr., Mr.†—University of California, Berkeley 4, Calif., U.S.A.
Carver, J. E., Mrs.*†—University of California, Berkeley 4, Calif., U.S.A.
Case, M. E., Dr.—Dept. of Botany, J. W. Gibbs Research Laboratory, Yale University, New Haven, Conn., U.S.A.
Caspar, A., Mr.—Box 85, Blandy Experimental Farm, Boyce, Va., U.S.A.
Caspar, A., Mrs.*—Box 85, Blandy Experimental Barm, Boyce, Va., U.S.A.
Caspari, E. W., Prof.—Shanklin Laboratory of Biology, Wesleyan University, Middletown, Conn., U.S.A.
Caspari, E. W., Mrs.*—48 Brainard Ave., Middletown, Conn., U.S.A.
Castiglioni, M. C., Dr.—Istituto di Genetica, Università di Milano, via Celoria 10, Milan, Italy
Catcheside, D. G., Prof.—Dept. of Microbiology, The University, Birmingham, England
Catcheside, D. G., Mrs.*—The Elms, Edgbaston Park Rd., Birmingham, England (Arnold)
Cavalcanti, A. G. L., Prof.—Universidade de Brasil, Avenida Antonio Carlos 40, Rio de Janeiro, Brazil
Cavalli-Sforza, L., Prof.—Dept. of Genetics, University of Parma, via Fatebenesorelle 18, Milan, Italy.
Celarier, R. P., Prof.—Oklahoma State University, Stillwater, Okla., U.S.A.
Chai, C. K., Dr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Chang, T.-D., Mr.—Dept. of Agronomy, University of Minnesota, St. Paul, Minn., U.S.A.
Chang, L., Mrs.*—230 Linden Ave., Ithaca, N.Y., U.S.A.
Chase, H. B., Prof.—Biology Dept., Brown University, Providence, R.I., U.S.A.
Chase, S., Dr.—DeKalb Agricultural Association Inc., Sycamore, Ill., U.S.A.
Chennaveeraiah, M. S., Dr.—Botanical Institute, University of Montreal, 4101 Sherbrooke St. E., Montreal 36, P.Q., Canada
Chiang, S.-M., Mr.—Box 105 Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Chiasson, L. P., Dr.—St. Francois Xavier University, Antigonish, N.S., Canada
Chiasson, L. P., Mrs.*—144 Greenwold, Antigonish, N.S., Canada
Childers, W. R., Dr.—Forage Crops Division, Central Experimental Farm, Ottawa, Ont., Canada
Childs, B., Dr.†—The Johns Hopkins Medical School, Baltimore, Md., U.S.A.
Chouinard, L., Dr.—Laval University, Quebec, P.Q., Canada
Chovnick, A., Prof.—University of Connecticut, Storrs, Conn., U.S.A.
Chavnick, A., Mrs.*—Wormwood Hill Rd., Storrs, Conn., U.S.A.
Chown, B., Dr.—University of Manitoba, Winnipeg, Man., Canada
Chu, E. H.-Y., Dr.—Dept. of Botany, Yale University, New Haven, Conn., U.S.A.
Clarke, C. A., Dr.—Dept. of Medicine, University of Liverpool, Liverpool, England
Clarke, C. A., Mrs.*—Dept. of Medicine, University of Liverpool, Liverpool, England
Clark, G. M., Prof.—Dept. of Zoology, University of Toronto, Toronto, Ont., Canada
Clark, R. D., Mr.—Experimental Farm, Lethbridge, Alta., Canada

Clausen, J., Dr.—Dept. of Plant Biology, Carnegie Institution of Washington, Stanford, Calif., U.S.A.
Clayberg, C. D., Dr.—Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, Conn., U.S.A.
Clayton, F. E., Prof.—Dept. of Zoology, University of Arkansas, Fayetteville, Ark., U.S.A.
Clayton, G., Dr.—A. R. C. Animal Genetics, West Mains Rd., Edinburgh 9, Scotland
Cleghorn, J. D., Mr.*—Redpath Museum, McGill University, Montreal, P.Q., Canada
Cleland, R. E., Prof.—Indiana University, Bloomington, Ind., U.S.A.
Clise, R. L., Mr.—Fenn College, Euclid Ave. at E. 24 St., Cleveland 15, Ohio, U.S.A.
Cocito, C., Dr.—Institute of Microbiology, Rutgers University, Highland Park, N.J., U.S.A.
Cockerham, C. C., Prof.—North Carolina State College, Raleigh, N.C., U.S.A.
Coe, E. H., Jr., Dr.—205 Curtis Hall, University of Missouri, Columbia, Mo., U.S.A.
Cohen, B. H., Dr.—The Johns Hopkins University, Baltimore, Md., U.S.A.
Cohen, B. H., Mr.*—3402 Devonshire Dr., Baltimore 15, Md., U.S.A.
Cohen, C., Dr.—Battelle Memorial Institute, 505 King Ave., Columbus, Ohio, U.S.A.
Cohen, J. A., Prof.—Medical Biological Laboratory of the RVO-TNO, 139 Lange Kleiweg, Rijswijk, Z. H., Netherlands
Cohn, M., Mrs.*—Biology Dept., The Johns Hopkins University, 34th & Charles Sts., Baltimore, Md., U.S.A.
Cohn, N. S., Dr.—Biology Dept., The Johns Hopkins University, 34th & Charles Sts., Baltimore, Md., U.S.A.
Cole, K., Dr.—Dept. of Biology & Botany, University of British Columbia, Vancouver 8, B.C., Canada
Colin, E. C., Prof.†—900 E. 83rd St., Chicago 19, Ill., U.S.A.
Colombo, G., Dr.—University of Padua, via Loredan 10, Padua, Italy
Comstock, R. E., Prof.—Animal Husbandry Dept., University of Minnesota, St. Paul 1, Minn., U.S.A.
Conger, A. D., Dr.†—Oak Ridge National Laboratory, P.O. Box Y, Oak Ridge, Tenn., U.S.A.
Conneally, P. M., Mr.*—Dept. of Genetics, University of Wisconsin, Madison, Wis., U.S.A.
Cook, A., Miss*—Genetics Dept., University of Wisconsin, Madison, Wis., U.S.A.
Cook, G. C., Dr.—719 N. Main St., South Bend, Ind., U.S.A.
Cook, G. C., Mrs.*—719 N. Main St., South Bend, Ind., U.S.A. (Claire & Gay)
Cook, R. C., Dr.—Population Reference Bureau Inc., 1507 M. St. N.W., Washington 5, D.C., U.S.A.
Cook, R. C., Mrs.*—1507 M. St. N.W., Washington 5, D.C., U.S.A.
Cooper, D. M., Dr.—Columbia University, New York, N.Y., U.S.A.
Corcoran, P. A., Miss*—332 Longwood Ave., Boston 15, Mass., U.S.A.
Cordeira, A., Dr.—Av. Paulo Gama, Porto Alegre, Rio Grande de Sul, Brazil
Cotter, W. B., Dr.—College of Charleston, 1351 Fairmount Ave., Mt. Pleasant, S.C., U.S.A.
Cotton, I., Dr.*—704 So. Lamar, Austin, Texas, U.S.A.
Cotton, J. E., Dr.—University of Texas, Austin, Texas, U.S.A.
Counce, S., Miss—O.Z.L. 231—Yale University, New Haven, Conn., U.S.A.
Cowan, J. R., Dr.—Farm Crops, Oregon State College, Corvallis, Ore., U.S.A.
Cowden, B., Mrs.*—1607 Park Ave., Baltimore, Md., U.S.A.
Cowden, R., Dr.—The Johns Hopkins University, Charles & 34th Sts., Baltimore, Md., U.S.A.
Cox, D., Mr.—Iowa State College, Ames, Iowa, U.S.A.
Coyle, J., Miss—Genetics Dept. University of Wisconsin, Madison 6, Wis., U.S.A.
Craft, W. A., Dr.—32 Curtiss Hall, Iowa State College, Ames, Iowa, U.S.A.
Craig, Mrs.*—Conara, 47 Prospect Hill Rd., Camberwell, Victoria, Australia
Craig, E. M., Dr.—Conara, 47 Prospect Hill Rd., Camberwell, Victoria, Australia
Crary, D. D., Mrs.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Creighton, H. B., Dr.—Botany Dept. Wellesley College, Wellesley 21, Mass., U.S.A.
Crittenden, L. B., Dr.—Creighton Brothers, Route 5, Warsaw, Ind., U.S.A.
Crosby, J. L., Dr.—Botany Dept. Durham Colleges, Science Laboratories, South Rd., Durham, England
Crow, J. F., Prof.—Genetics Dept. University of Wisconsin, Madison 6, Wis., U.S.A.
Crow, J. F., Mrs.*—Genetics Dept. University of Wisconsin, Madison 6, Wis., U.S.A. (Frank, Laura, & Catherine)
Crowe, L.-K., Dr.—Radcliffe Graduate Center, 6 Ash St., Cambridge, Mass., U.S.A.
Cuany, R. L., Dr.—Biology Dept. Brookhaven National Laboratory, Upton, N.Y., U.S.A.

Curtis, E., Mrs.—Hospital for Sick Children, 555 University Ave., Toronto 2, Ont. Canada
Curtis, R., Dr.—Dominion Experimental Farm, Fredericton, N.B., Canada
Curtiss, R. III., Mr.—Biology Dept., Brookhaven National Laboratory, Upton, N.Y., U.S.A.

Daly, K., Mr.—Dept. of Plant Breeding, Cornell University, Ithaca, N.Y., U.S.A.
D'Amato, F., Prof.—Instituto di Botanica della Universita, viale Fra Ignazio 13, Cagliari, Italy
Danon-Galland, M., Dr.—Weismann Institute of Science, Nevah Weizmann, Rehovoth, Israel
Dansereau, P., Dr.—Université de Montréal, Institut Botanique, 4101 est, rue Sherbrooke, Montreal 36, P.Q., Canada
Dansereau, P., Mrs.*—76 Maplewood Ave., Montreal, P.Q., Canada
Dark, S. O. S., Dr.—Cotton Breeding Section, Ministry of Agriculture, Research Division, Khartoum North, Sudan
Dark, S. O. S., Mrs.*—Shambat, P.O. Box 30, Khartoum North, Sudan
Dasananda, S., Dr.—Biology Dept., Chulalongkorn University, Bangkok, Thailand
Da Silva, A. R., Dr.—Instituto Agronomico, Do Sul, Pelotas, Brazil
Dassat, P., Prof.—Observatorio Genetica Animale, Pastrengo 28, Torino, Italy
Davern, C. I., Mr.—California Institute of Technology, San Pasqual, Pasadena, Calif., U.S.A.
Davis, A., Miss—Dept. of Zoology, University of Chicago, Chicago, Ill., U.S.A.
Davis, H. M., Mr.—Brookhaven National Laboratory, Upton, N.Y., U.S.A.
Dawson, C. D. R., Dr.—A. L. Tozer, Ltd., Pyports, Church St., Cobham, Surrey, England
De, D. N., Mr.—Dept. of Botany, Columbia University, New York 27, N.Y., U.S.A.
Decker, H. L., Miss*—Dept. of Genetics, University of Wisconsin, Madison, Wis., U.S.A.
Degenhardt, K. H., Dr.—Institut für Humangenetik der Universität, Waldeyerstr. 27, Münster, Westfalen, Germany
De Giovanni, R. F., Dr.—Dept. of Biochemistry, Columbia University, New York, N.Y., U.S.A.
De Jamblinne de Meux, A., Dr.—Centre de Biologie Forestière, Bokryk, Winterslag-Genk, Limbourg, Belgium
DeLamater, E. D., Prof.†—School of Medicine, University of Pennsylvania, Philadelphia, Pa., U.S.A.
DeMarinis, F., Prof.—Dept. of Biology, Fenn College, Cleveland 15, Ohio, U.S.A.
Demarly, Y., Dr.—Institut National de la Recherche Agronomique, 1 Ave., Division Leclerc, Saint Cyr, (S & O) France
Demerec, M., Dr.—Dept. of Genetics, Carnegie Institution of Washington, Cold Spring Harbour, N.Y., U.S.A.
De Moura Pedreira, C., Dr.—Faculdade de Filosofia da Universidade da Bahia, Salvador, Brazil
Dempster, E. R., Prof.—Dept. of Genetics, University of California, Berkeley 4, Calif., U.S.A.
Denhardt, D. T., Mr.—Swarthmore College, Swarthmore, Pa., U.S.A.
Derlogia, V., Mme.—Institut de Recherches Fototecniques, Bucarest, Roumanie
Dermen, H., Dr.—Plant Industry, U.S.D.A. Beltsville, Md., U.S.A.
De Sampayo, T. J., Dr.†—Dept. of Botany, University of Illinois, Ill., U.S.A.
De Sampayo, T. J., Mrs.*†—Dept. of Botany, University of Illinois, Ill., U.S.A.
De Serres, F. J., Dr.—Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Dettmers, A. E., Mr.—Dept. of Animal Husbandry, University of Minnesota, St. Paul 1, Minn., U.S.A.
De Vilmorin, R., Dr.—Vilmorin-Andrieux, Plant Breeders, Verrières-le-Buisson, (S & O), France
Dhawan, C., Mr.*—Agronomy Dept., Purdue University, Lafayette, Ind., U.S.A.
Dickerson, G. E., Dr.—Kimber Farms Inc., Niles, Calif., U.S.A.
Dickerson, G. E., Mrs.*—R1, Box 17, Olive Ave., Irvington, Calif., U.S.A. (Dean)
Dickie, M. M., Dr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Dickinson, A. G., Dr.—Animal Breeding Research Organization, 6 South Oswald Rd., Edinburgh, Scotland
Dickson, M. H., Mr.—Horticultural Dept., Ontario Agricultural College, Guelph, Ont., Canada
Di Pasquale, A., Dr.—Instituto di Genetica, Universita de Milano, Milan, Italy
Dissosway, C. F. R., Miss—Amherst College, Amherst, Mass., U.S.A.

Doane, W. W., Mrs.—Osborn Zoology Laboratory, Rm. 215, Yale University, New Haven, Conn., U.S.A.
Dobzhansky, T., Dr.—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Dobzhansky, T., Mrs.*—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Dodds, K. S., Dr.—John Innes Horticultural Institution, Bayfordbury, Hertford, Herts., England
Dodson, E. O., Dr.—University of Ottawa, Ottawa 2, Ont., Canada
Doidge, E., Miss*—University of Toronto, Toronto, Ont., Canada
Donald, H. P., Dr.—A.R.C. Animal Breeding Research Organization, 6 South Oswald Rd., Edinburgh 9, Scotland
Donovan, L., Dr.—Forage Crops Division, Central Experimental Farm, Ottawa, Ont., Canada
Dore, W. G., Dr.*—Botany & Plant Pathology Laboratory, Dept. of Agriculture, Science Service, Ottawa, Ontario, Canada
Dorman, K. W., Mr.—U.S. Forest Service, Box 2570, Asheville, N.C., U.S.A.
Doucet, R., Mr.*—University of Montreal, Montreal, P.Q., Canada
Doudney, C. O., Dr.†—Genetics Dept., M.D. Anderson Hospital, Houston, Texas, U.S.A.
Douka, S. H., Prof.—Ukrainian Scientific Research Institute of Horticulture, 8 Agronomicheskij Kiev, YS, U.S.S.R.
Downes, J. A., Dr.—Division of Entomology, Canada Dept. of Agriculture, 370 Science Service Bldg., Ottawa, Ont., Canada
Dowrick, G. J., Dr.—John Innes Horticultural Institute, Bayfordbury, Hertford, Herts., England
Doyle, R. J., Mr.—2930 Wayne State University, Detroit Institute of Cancer Research, Detroit, Mich., U.S.A.
Drolsom, P. N., Dr.—Agonomy Dept., University of Wisconsin, Madison, Wis., U.S.A.
Dudgeon, E., Miss—Southern Illinois University, Carbondale, Ill., U.S.A.
Dunn, L. C., Prof.—Columbia University, New York, N.Y., U.S.A.
Durrant, A., Dr.—Dept. of Agricultural Botany, University College of Wales, Aberystwyth, Wales
Duvick, D., Dr.—Pioneer Hi-Bred Corn Company, Johnston, Iowa, U.S.A.
Duvick, D., Mrs.*—Pioneer Hi-Bred Corn Company, Johnston, Iowa, U.S.A.

Eaton, O. N., Dr.—Microbiological Associates Inc., Hyattsville, Md., U.S.A.
Eaton, O. N., Mrs.*—4320 Clagett Rd., Hyattsville, Md., U.S.A.
Eberhard, B., Dr.—Box 704, Princeton University, Princeton, N.J., U.S.A.
Ebersold, W. T., Dr.—Biological Laboratories, Harvard University, Cambridge, Mass., U.S.A.
Ebersold, W. T., Mrs.*—Biological Laboratories, Harvard University, Cambridge, Mass. U.S.A.
Echols, R. M., Dr.—U.S. Forest Service, Southern Institute of Forest Genetics, Gulfport, Miss., U.S.A.
Edwards, J. M., Dr.—Birmingham University, Medical School, Birmingham, England
Edwards, R. G., Dr.—National Institute for Medical Research, Mill Hill, London N.W. 7, England
Edwards, R. G., Mrs.*—The Ridgeway, Mill Hill, London N.W. 7, England
Egelhaaf, A., Dr.—Max-Planck-Institut für Biologie, Spemannestrasse 34, Tübingen, Germany
Ehling, U., Dr.—Max-Planck-Institut für verg. Erbbiologie, und Erbpathologie, Berlin-Dahlem, Germany
Eid, S. E., Dr.†—Faculty of Agriculture, The University, Shatby, Alexandria, Egypt
Eigsti, E. Mr.*—6800 S. Stewart, Chicago, Ill., U.S.A.
Eigsti, N., Mr.*—6800 S. Stewart, Chicago, Ill., U.S.A.
Eigsti, O., Prof.—Chicago Teacher's College, Chicago, Ill., U.S.A.
Eigsti, O., Mrs.*—6800 S. Stewart, Chicago, Ill., U.S.A.
Ellis, J. R., Dr.—University of Manchester, Oxford Rd., Manchester, England
Emerson, S., Prof.—Biology Division, California Institute of Technology, Pasadena, Calif., U.S.A.
Emery, G. C., Mr.—Pennsylvania State University, State College, Pa., U.S.A.
Emsweller, S. L., Dr.—U.S.D.A., Plant Industry Station, Beltsville, Md., U.S.A.
Enesco, M., Dr.*—Dept. of Anatomy, McGill University, Montreal 2, P.Q., Canada
Englert, D. C., Mr.*—Purdue University, Lafayette, Ind., U.S.A.
Enikeev, K. K., Dr.—Moscow Experimental Station of Fruit Cultures, Moscow, Birulewo, U.S.S.R.
Erben, K., Dr.—(22c) Köln-Brück, Pohlstadtsweg 426, West Germany
Esser, K., Dr.—Botanisches Institut, Universität Köln, Köln-Lindenthal, Germany

Evans, A. M., Dr.—Welsh Plant Breeding Station, Aberystwyth, Wales
Evans, D. H., Dr.—Defence Research, Kingston Laboratory, Box 123, Kingston, Ont., Canada
Evans, H. J., Dr.—Medical Research Council, Radiobiological Unit, A.E.R.E., Harwell, Didcot Berks., England
Evans, L. E., Dr.—Plant Science Dept., University of Manitoba, Winnipeg, Man., Canada

Faberge, A. C., Dr.—Zoology Dept., University of Texas, Austin 12, Texas, U.S.A.
Fahmy, O. G., Dr.—Chester Beatty Research Institute, Pollards Wood Research Station, Nightingales Lane, Chalfont St. Giles, Bucks., England
Failla, G., Prof.—College of Physicians & Surgeons, Columbia University, New York 32, N.Y., U.S.A.
Failla, G., Mrs.*—630 W. 168th St., New York 32, N.Y., U.S.A.
Falconer, D. S., Dr.—Institute of Animal Genetics, West Mains Rd., Edinburgh 9, Scotland
Falek, A., Dr.—N.Y. State Psychiatric Institute, Columbia University College of Physicians & Surgeons, New York, N.Y., U.S.A.
Falk, R., Dr.†—Dept. of Zoology, The Hebrew University of Jerusalem, Jerusalem, Israel
Fankhauser, G., Prof.—Dept. of Biology, Princeton University, Princeton, N.J., U.S.A.
Fantini, A. A. Mr.*—Dept. of Botany, Columbia University, New York 27, N.Y., U.S.A.
Farnsworth, G. M., Dr.—Kimber Farms Inc., Niles, Calif., U.S.A.
Farthing, B. R., Dr.—North Carolina State College, Raleigh, N.C., U.S.A.
Fechheimer, N., Dr.—Plumb Hall, Ohio State University, Columbus 10, Ohio, U.S.A.
Fechheimer, N., Mrs.*—Plumb Hall, Ohio State University, Columbus 10, Ohio, U.S.A
Feinbrun, N., Dr.†—Hebrew University, Jerusalem, Israel
Feist, G. V., Dr.—702 Professional Bldg., Kansas City 6, Mo., U.S.A.
Fejer, S. O., Dr.—Grasslands Division, D.S. & I.R., Palmerston North, New Zealand
Fenaroli, L., Prof.—Stazione Sperimentale Maiscoltura, Bergamo, Italy
Ferguson, P., Mr.—Dept. of Genetics, McGill University, Montreal 2, P.Q., Canada
Fernandez, C. E., Mr.—Dept. of Horticulture, Rutgers University, New Brunswick, N.J., U.S.A.
Fincham, J. R. S., Dr.—University of Leicester, Leicester, England
Finlay, K. W., Dr.—University of Adelaide, Adelaide, Australia
Fitch, N., Dr.—Cornell Medical School, 430 W. 118th St., New York, N.Y., U.S.A.
Florian, S. F., Dr.*—Cancer Institute, Saskatoon, Sask., Canada
Flory, W. S., Prof.—Blandy Experimental Farm, Boyce, Va., U.S.A.
Foothills, J. C.—Tennessee Intermountain College, Nazareth, Tenn., U.S.A.
Forest, B., Dr.—Ferme Experimentale, Ste Anne de la Pocatière, P.Q., Canada
Forrest, H. S., Dr.—Zoology Dept., University of Texas, Austin 12, Texas, U.S.A.
Forsthoefel, P. F., Dr.—Lansing-Reilly Hall, University of Detroit, Detroit 21, Mich., U.S.A.
Foster, M., Dr.†—Dermatology Section, Dept. of Medicine, Yale University, New Haven, Conn., U.S.A.
Fowler, D., Mr.—Ontario Dept. of Lands & Forest, Southern Research Station, Maple, Ont., Canada
Fox, A. S., Prof.—Michigan State University, East Lansing, Mich., U.S.A.
Fraccaro, M., Dr.—State Institute for Human Genetics, Uppsala, Sweden
François, J., Prof.†—Place de Smet de Naeyer 2015, Gand, Belgium
Frandsen, K. J., Dr.—D.L.F. & F.D.B. Plant Breeding Stations, Otoftegaard, Taastrup, Denmark
Frankel, O. H., Dr.—Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T., Australia
Frankel, O. H., Mrs.*—P.O. Box 109, Canberra, A.C.T., Australia
Fraser, A. S., Dr.—Zoology Dept., Sydney University, Sydney, N.S.W., Australia
Fraser, F. C., Dr.—Dept. of Genetics, McGill University, Montreal 2, P.Q., Canada
Fraser, F. C., Mrs.*—124 Percival Ave., Montreal, P.Q., Canada (Norah, Noel, Alan)
Fraser, G. R., Dr.—Galton Laboratory, University College, Gower St., London W.C.1, England
Fredeen, H., Dr.—Canada Dept. of Agriculture, Experimental Farm, Lacombe, Alta., Canada
Freeman, A. E., Dr.—Iowa State College, Ames, Iowa, U.S.A.
Freeman, J., Asst.*—University of Illinois, Urbana, Ill., U.S.A.
Freese, E., Dr.—Biological Laboratory, Harvard University, Cambridge, Mass., U.S.A.
Freese, E., Mrs.*—130 Lakeview Avenue, Cambridge, Mass., U.S.A. (Catherine)
Freire-Maia, A., Prof.†—Laboratory of Genetics, University of Paraná, Curitiba, Parana, Brazil
Freire-Maia, N., Prof.—Laboratory of Genetics, University of Paraná, Curitiba, Parana, Brazil

Frezal, J., Dr.—Clinique de Génétique Mèdicale, Hospital des Enfants Malades de Sèvres 149, Paris 15, France
Frizzi, G., Prof.—Instituto di Zoologia, Palazzo Botta, Pavia, Italy
Frost, J. N., Dr.†—University of California, Berkeley 2, Calif., U.S.A.
Frye, S. H., Miss—Indiana University, Bloomington, Ind., U.S.A.
Fuad, J. A., Mr.—Agronomy Dept., University of Minnesota, St. Paul 8, Minn., U.S.A.
Fuerst, R., Dr.—Texas Woman's University, Denton, Texas, U.S.A.
Fuller, J. L., Dr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Fuscaldo, K., Miss—Dept. of Zoology, Michigan State University, East Lansing, Mich., U.S.A.
Fussell, C. P., Miss—Biology Dept., Brookhaven National Lab., Upton, N.Y., U.S.A.

Gabelman, W. H., Dr.—31 B University Houses, University of Wisconsin, Madison, Wis., U.S.A.
Gabelman, W. H., Mrs.*—31 B University Houses, University of Wisconsin, Madison, Wis., U.S.A.
Gajewski, W., Prof.—Botanic Gardens, University of Warsaw, al Ujazdowskie 4, Warsaw, Poland
Galinsky, I., Dr.—Biochemical Research Foundation, S. Academy, Newark, Del., U.S.A.
Galinsky, I., Mrs.*—Biochemical Research Foundation, S. Academy, Newark, Del., U.S.A. (Claude & Douglas)
Gall, J., Dr.†—Dept. of Zoology, University of Minnesota, Minneapolis, Minn., U.S.A.
Gamble, E. E., Dr.—Field Husbandry Dept., Ontario Agriculture College, Guelph, Ont., Canada
Garber, E., Dr.—Dept. of Botany, University of Chicago, Chicago, Ill., U.S.A.
Gardner, C. O., Dr.—University of Nebraska, Lincoln, Neb., U.S.A.
Gardner, E. J., Prof.—Utah State University, Logan, Utah, U.S.A.
Garnjobst, L., Dr.—Rockefeller Institute, 66th St. & York Ave., New York, N.Y., U.S.A.
Gartler, S., Dr.—School of Medicine, University of Washington, Seattle, Wash., U.S.A.
Gates, A. H., Mr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Gaul, H., Dr.—Max-Planck-Institut für Zuchtungsforchung, Köln-Vogelsang, Germany
Gay, H., Dr.*—Carnegie Institution, Cold Spring Harbor, N.Y., U.S.A.
Gedda, L., Prof.—Istituto di Genetica Medica e Gemmellolgia "Gregorio Mendel," Rome, Italy
Geerts, S. J., Dr.—Laboratory of General Biology & Genetics, Nymegen, Netherlands
Gerald, P., Dr.—Children's Medical Center, Harvard Medical School, Boston, Mass., U.S.A.
Gershowitz, H., Dr.—University of Michigan, Ann Arbor, Mich., U.S.A.
Gerstel, D. U., Prof.—North Carolina State College, Raleigh, N.C., U.S.A.
Gerstel, D. U., Mrs.*—North Carolina State College, Raleigh, N.C., U.S.A. (David & Naomi)
Ghany, F., Mr.*—1001 Sherbrooke St. W., Montreal, P.Q., Canada
Gibbons, I. R., Dr.—Biological Laboratories, Harvard University, Cambridge 38, Mass., U.S.A.
Giesbrecht, J. E., Dr.—Experimental Farm, Morden, Man., Canada
Giesbrecht, J. E., Mrs.*—156-5th St., Morden, Man., Canada (Karl, Elizabeth & Dora)
Giles, B. D., Miss*—Biology Dept., Western Reserve University, Cleveland, Ohio, U.S.A.
Giles, J. A., Dr.*—Sociedad Nacional Agraria, Lima, Peru
Giles, J. A., Mrs.*—Sociedad Nacional Agraria, Lima, Peru
Giles, N. H., Prof.—Josiah Willard Gibbs Research Laboratory, Yale University, New Haven, Conn., U.S.A.
Gilman, J., Dr.—Ontario Veterinary College, Guelph, Ont., Canada
Gini, C., Prof.—Italian Society of Genetics & Eugenics, University of Rome, via Adige 39, Rome, Italy
Giordano, A., Dr.—University of Milan, via F. Sforza 38, Milan, Italy
Gladstones, J. S., Mr.—Institute of Agriculture, University of Western Australia, Nedlands, Western Australia
Glass, H. B., Prof.—The Johns Hopkins University, Baltimore, Md., U.S.A.
Glass, H. B., Mrs.*—339 Rosebank Ave., Baltimore, Md., U.S.A.
Glassman, E., Dr.—City of Hope Medical Center, Duarte, Calif., U.S.A.
Glavinitch, R., Prof.—Faculté Forestière, Kn. Viseslavat, Belgrade, Yugoslavia
Gloor, H., Prof.—Dept. of Genetics, University of Leiden, Leiden, Netherlands
Gloor, H., Mrs.*—Witte Singel 57, Leiden, Netherlands

Glouchtchenko, I., Prof.—Institute of Genetics, Academy of Sciences, Moscow, U.S.S.R.
Godfrey, G. F., Dr.—Honegger Breeder Hatchery, Forrest, Ill., U.S.A.
Godfrey, G. F., Mrs.*—Honegger Breeder Hatchery, Forrest, Ill., U.S.A.
Gokcora, H., Dr.—College of Agriculture, Ankara University, Ankara, Turkey (Zula & Haluk)
Goldberg, R. J., Dr.—Chicago Teacher's College, Chicago, Ill., U.S.A.
Goldschmidt, E., Dr.—Dept. of Zoology, Hebrew University, Jerusalem, Israel
Goodgal, S. H., Dr.—The Johns Hopkins University, Baltimore 5, Md., U.S.A.
Goodgal, S. H., Mrs.*—615 North Wolfe St., Baltimore 5, Md., U.S.A. (Dana & Charles)
Goodman, R., Dr.—Barnard College, Columbia University, New York 27, N.Y., U.S.A.
Goodman, S., Dr.—Columbia University, New York 27, N.Y., U.S.A.
Goodsell, S. F., Dr.—Pioneer Hi-Bred Corn Company, Des Moines, Iowa, U.S.A.
Goodwin, K., Dr.—Kimber Farms Inc., Niles, Calif., U.S.A.
Gopal-Ayengar, A. R., Dr.—Atomic Energy Establishment, Biology Division, Indian Cancer Research Centre, Bombay 12, India
Goplen, B. E. P., Dr.—Forage Crops Section, Canada Agriculture Research Laboratory, Saskatoon, Sask., Canada
Gordon, M. J., Prof.—Dept. of Dairy, Michigan State University, East Lansing, Mich., U.S.A.
Gordon, M. J., Mrs.*—Dept. of Dairy, Michigan State University, East Lansing, Mich., U.S.A. (Lynne & Jean)
Gordon, M., Dr.—American Museum Natural History, New York, N.Y., U.S.A.
Goulden, C. H., Dr.—Dept. of Agriculture, Central Experimental Farm, Ottawa, Ont., Canada
Goux, J.-M., Dr.—Laboratoire de Génétique, Université de Paris, Paris, France
Gowans, C. S., Dr.—Botany Dept., University of Missouri, Columbia, Mo., U.S.A.
Gowen, J. W., Dr.—Dept. of Genetics, Iowa State College, Ames, Iowa, U.S.A.
Gowen, J. W., Mrs.*—Dept. of Genetics, Iowa State College, Ames, Iowa, U.S.A.
Graf, G., Dr.—Zoology–Comparative Anatomy, University of Zurich, Zurich, Switzerland
Graham, K., Dr.—Plant Pathology Laboratory, Fredericton, N.B., Canada
Grahn, D., Dr.—Argonne National Laboratory, Lemont, Ill., U.S.A.
Grant, C., Dr.—Kimber Farms Inc., Niles, Calif., U.S.A.
Grant, W. F., Dr.—Dept. of Genetics, Macdonald College, McGill University, Ste. Anne de Bellevue, P.Q., Canada
Grant, W. F., Mrs.*—Macdonald College, McGill University, Ste. Anne de Bellevue, P.Q., Canada
Graumann, H., Dr.—U.S.D.A., Plant Industry Station, Beltsville, Md., U.S.A.
Graumann, H., Mrs.*—7200 Flower Ave., Takoma Park, Md., U.S.A. (DeEstye)
Greb, R. J., Prof.—South Dakota State College, State College, S.D., U.S.A.
Greb, R. J., Mrs.*—State College, S.D., U.S.A. (Helen)
Green, E. L., Dr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Green, M. C., Dr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Green, M. M., Dr.—Dept. of Genetics, University of California, Davis, Calif., U.S.A.
Green, M. M., Mrs.*—Dept. of Genetics, University of California, Davis, Calif., U.S.A.
Greenberg, R., Miss*—University of Wisconsin, Madison, Wis., U.S.A.
Greenshields, J. E. R., Dr.—Central Experimental Farm, Ottawa, Ont., Canada
Greer, S., Dr.—Columbia College of Physicians & Surgeons, 630 W. 168th St., New York, N.Y., U.S.A.
Gregg, T., Dr.—University of Texas, Austin, Texas, U.S.A.
Gregory, D., Miss—National Science Foundation, Washington, D.C., U.S.A.
Gregory, K., Dr.†—A.R.S.-U.S.D.A., 917 Ferndale Rd., Lincoln, Neb., U.S.A.
Gregory, P. W., Prof.—Dept. of Animal Husbandry, University of California, Davis, Calif., U.S.A.
Grell, E. H., Dr.—Dept. of Biology, California Institute of Technology, Pasadena, Calif., U.S.A.
Grell, E. H., Mrs.—1201-East California St., Pasadena 4, Calif., U.S.A.
Griffiths, D. J., Dr.—Welsh Plant Breeding Station, Aberystwyth, Wales
Grosch, D. S., Prof.—Dept. of Genetics, North Carolina State College, Raleigh, N.C., U.S.A.
Grosch, D. S., Mrs.*—Dept. of Genetics, North Carolina State College, Raleigh, N.C., U.S.A. (Laura)
Gross, S. R., Dr.—Rockefeller Institute, New York, N.Y., U.S.A.
Groszmann, A., Prof.—Universidade Rural, Rio de Janeiro, D.F., Brazil
Groszmann, A., Mrs.*—Universidade Rural, Rio de Janeiro, D.F., Brazil

Growich, J. A., Jr., Dr.—Lederle Laboratories, American Cyanamid Co., Pearl River, N.Y., U.S.A.
Grun, P., Dr.—Dept. of Botany, Pennsylvania State University, University Park, Pa., U.S.A.
Guthrie, R., Dr.—University of Buffalo, Williamsville, N.Y., U.S.A.
Guzman, O., Dr.—Asociacion Internacional de Profesionistas, Av. Juarez 104–24, Mexico City, Mexico
Gwynn, E., Mr.—Washington College, Chestertown, Md., U.S.A.
Gyorffy, B., Dr.†—Herman Otto ut 15, Budapest, Hungary

Haas, F. L., Dr.—University of Texas M.D. Anderson Hospital & Tumor Institute, Houston, Texas, U.S.A.
Haberlandt, W. F., Dr.—Institut für Humangenetik der Universität, Munster, Germany
Hackel, E., Dr.—Dept. of Natural Science, Michigan State University, East Lansing, Mich., U.S.A.
Hacker, F., Dr.—33 Teller Ave., Beacon, N.Y., U.S.A.
Haddox, C H., Dr.—Einstein Northern Medical Center, Philadelphia, Pa., U.S.A.
Hadley, H., Dr.*—University of Illinois, Champaign, Ill., U.S.A.
Hadorn, E., Prof.—Zoological Institute, University of Zurich, Zurich, Switzerland
Hagemann, R., Dr.†—Institut für Kulturplanzenforschung, Gatersleben, Germany D.D.R.
Hagy, G. W., Dr.—Dept. of Biology, Brown University, Providence, R.I., U.S.A.
Hair, J. B., Dr.—Botany Division, D.S.I.R., Christchurch, New Zealand
Hale, H. B., Dr.—Provincial Hospital, Lancaster, N.B., Canada
Hamerton, J. L., Mr.—British Museum (Natural History), Cromwell Rd., London, S.W.7, England
Hamilton, D. G., Dr.—Cereal Crops Division, Dept. of Agriculture, Experimental Farm, Ottawa, Ont., Canada
Hamilton, H., Dr.*—American Society of Agronomy, 2702 Monroe St., Madison. Wis.. U.S.A.
Hamilton, H. H., Mrs.*—562 Fraser Ave., Ottawa, Ont., Canada
Hammons, H. G., Mrs.—*Eugenics Quarterly*, American Eugenics Society, 230 Park Ave., New York 17, N.Y., U.S.A. (Patricia)
Hanly, E. W., Dr.—University of Texas, Austin, Texas, U.S.A.
Hanna, B. L., Dr.—Medical College of Virginia, Richmond 19, Va., U.S.A.
Hannah, A. E., Dr.—Cereal Crops Division, Canada Dept. of Agriculture, Ottawa, Ont., Canada
Hanson, C. H., Dr.†—Dept. of Field Crops, North Carolina State College, Raleigh, N.C., U.S.A.
Haq, M. S., Dr.—8 Bakshi Bazar Rd., Dacca, East Pakistan, Pakistan
Hardy, L. B., Dr.—Roswell Park Memorial Institute, Biological Station, Springville, N.Y., U.S.A.
Harlan, J., Prof.—Oklahoma State University, Stillwater, Okla., U.S.A.
Harlan, J., Mrs.*—Route 2, Stillwater, Okla., U.S.A. (Sue, Harry, Sherry & Richard)
Harland, S., Prof.—University of Manchester, Manchester, England
Harney, P., Miss—Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Harrington, J. B., Dr.—FAO of United Nations, FAO, Rome, Italy
Harrington, J. B., Mrs.*—FAO of United Nations, FAO, Rome, Italy
Harris, H., Dr.—Dept. of Biochemistry, London Hospital Medical College, London E.1, England
Harrison, G. A., Dr.—University of Liverpool, Liverpool, England
Harte, C., Prof.—Universität zu Köln, Institut für Entwicklungsphysiologie, Gyrhofstrasse, 17, Köln, Germany
Hartman, P. E., Dr.—Dept. of Biology, The Johns Hopkins University, Baltimore 18, Md., U.S.A.
Hartman, P. E., Mrs.*—Dept. of Biology, The Johns Hopkins University, Baltimore 18, Md., U.S.A. (Paul)
Harvald, B., Dr.—University Institute for Human Genetics, Cophenhagen, Denmark
Hasimoto, H., Dr.—Matsumoto Branch, Sericultural Experiment Station, M.A.F., Matsumoto, Japan
Haskins, F. A., Dr.—Dept. of Agronomy, University of Nebraska, Lincoln, Neb., U.S.A.
Hatton, M. E., Dr.—Dept. of Zoology, University of Toronto, Toronto, Ont., Canada

Hay, M. C., Dr.*—3724 Clarke Dr., Vancouver, B.C., Canada
Hazel, L. N., Prof.—Iowa State College, Ames, Iowa, U.S.A.
Hebb, C., Dr.—Detroit Institute of Cancer Research, Detroit, Mich., U.S.A.
Hecht, A., Prof.—State College of Washington, Pullman, Wash., U.S.A.
Heed, W., Dr.—Dept. of Zoology, University of Texas, Austin, Texas, U.S.A.
Heidenthal, G., Dr.—Russel Sage College, Troy, N.Y., U.S.A.
Heimburger, C., Dr.—Ontario Dept. of Lands & Forests, Southern Research Station, Maple, Ont., Canada
Heinrichs, D. H., Dr.—Experimental Farms Service, Swift Current, Sask., Canada
Helgason, S. B., Dr.—University of Manitoba, Winnipeg 9, Man., Canada
Henderson, N. E., Miss*—Zoology Department, University of Toronto, Toronto, Ont., Canada
Henley, W., Dr.*—College of Physicians & Surgeons, Columbia University, New York 62, N.Y., U.S.A.
Herskowitz, I. H., Dr.—St. Louis University, St. Louis, Mo., U.S.A.
Heslot, H., Dr.†—Inst. National Agronomique, 16 rue Claude Bernard, Paris V, France
Hessler, A., Mrs.—Dept. of Zoology, University of Chicago, Chicago, Ill., U.S.A.
Heston, W. E., Dr.—National Cancer Institute, Béthesda 14, Md., U.S.A.
Hetzer, H. O., Dr.—U.S.D.A., Agriculture Research Center, Beltsville, Md., U.S.A.
Hetzer, H. O., Mrs.*—409 Northwest Dr., Silver Springs, Md., U.S.A.
Hexter, W. M., Dr.—Biology Dept., Amherst College, Amherst, Mass., U.S.A.
Hexter, W. M., Mrs.*—Biology Dept., Amherst College, Amherst, Mass., U.S.A. (James)
Heyne, E. G., Dr.—Agronomy Dept., Kansas State College, Manhattan, Kan., U.S.A.
Heyne, E. G., Mrs.*—Agronomy Dept., Kansas State College, Manhattan, Kan., U.S.A. (George, Kathryn, Robert & David)
Hickman, C. G., Dr.—Central Experimental Farm, Ottawa, Ont., Canada
Hicks, A. F., Jr., Dr.—Dept. of Poultry Science, University of British Columbia, Vancouver, B.C., Canada
Higgins, J. V., Mr.—Dight Institute, University of Minnesota, Minneapolis, Minn., U.S.A.
Higgins, J. V., Mrs.*—Dight Institute, University of Minnesota, Minneapolis, Minn., U.S.A.
Highkin, H. R., Dr.—Division of Biology, California Institute of Technology, Pasadena, Calif., U.S.A.
Hildemann, W. H., Dr.—University of California, Los Angeles, Calif., U.S.A.
Hill, B. F., Dr.—National Research Council (U.S.A.), Washington, D.C., U.S.A.
Hillman, R., Dr.—Zoological Laboratory, University of Pennsylvania, Philadelphia, Pa., U.S.A.
Hinton, C. W., Dr.—Dept. of Zoology, University of Georgia, Athens, Ga., U.S.A.
Hinton, C. W., Mrs.*—Dept. of Zoology, University of Georgia, Athens, Ga., U.S.A.
Hirota, Y., Mr.—Dept. of Genetics, University of Wisconsin, Madison 6, Wis., U.S.A.
Hiroyoshi, T., Dr.—Dept. of Entomology, University of Kansas, Lawrence, Kan., U.S.A.
Hirschfeld, W. K., Prof. Dr.—Zootechnisch Institute, Utrecht, Netherlands
Hirschhorn, K., Dr.—N.Y.U. Post-Graduate Medical School, New York 16, N.Y., U.S.A.
Hittle, C. N., Dr.*—University of Illinois, Champaign, Ill., U.S.A.
Hochman, B., Dr.—Dept. of Genetics, University of Utah, Salt Lake City, Utah, U.S.A.
Hogsett, M. L., Mr.—Heisdorf & Nelson Farms, Inc., Kirkland, Wash., U.S.A.
Hollaender, A., Dr.—Oak Ridge National Laboratory, Biology Division, Oak Ridge, Tenn., U.S.A.
Hollander, W. F., Prof.—Iowa State College, Ames, Iowa, U.S.A.
Hollands, K. G., Mr.—Poultry Division, Dept. of Agriculture, Central Experimental Farm, Ottawa, Ont., Canada
Hollinshead, M., Dr.*—Seton Hall College of Medicine & Dentistry, Medical Center, Jersey City, N.J., U.S.A. (Richard)
Holloway, B. W., Dr.—Dept. of Bacteriology, University of Melbourne, Carlton N. 3, Victoria, Australia
Holmsen, T., Mr.—Dept. of Horticulture, Rutgers University, New Brunswick, N.J., U.S.A.
Holst, M., Mr.—Dept. of Northern Affairs and Natural Resources, Petawawa Forest Experimental Station, Chalk River, Ont., Canada
Holt, S. B., Dr.—Galton Laboratory, University College, Gower St., London, W.C.1, England
Holt, S. J., Miss*—Galton Laboratory, University College, Gower St., London W.C.1, England
Homsher, P. J., Jr., Mr.—Pennsylvania State University, State College, Pa., U.S.A.
Honeyman, M., Dr.—National Cancer Institute, Bethesda, Md., U.S.A.
Honeyman, M., Mrs.*—9000 Wisconsin Ave., Bethesda, Md., U.S.A.

Hooven, N. W., Mr.—ARS Animal Husbandry Research Div., Dairy Cattle Research Branch, Beltsville, Md., U.S.A.
Horovitz, S., Ing. Agr.—Universidad Central de Venezuela, Maracay, Venezuela
Horowitz, N. H., Prof.—California Institute of Technology, Pasadena, Calif., U.S.A.
Hough, L. F., Dr.†—Dept. of Horticulture, Rutgers University, New Brunswick, N.J., U.S.A.
House, L., Dr.—Purdue University, W. Lafayette, Ind., U.S.A.
Hovanitz, W., Dr.†—1140 W. Orange Grove Ave., Arcadia, Calif., U.S.A.
Howard, A., Dr.—Research Unit in Radiobiology, Mount Vernon Hospital, Northwood, Middlesex, England
Howard-Flanders, P., Dr.—Donner Laboratory, University of California, Berkeley 3, Calif., U.S.A.
Howell, W. E., Dr.—University of Saskatchewan, Saskatoon, Sask., Canada
Hruby, K., Prof.—Genetics Institute, Charles University, Vinicna 5, Praha 2, Czechoslovakia.
Hsia, D. Y-Y, Dr.—Northwestern University, Chicago 14, Ill., U.S.A.
Hsia, D.Y-Y, Mrs.*—707 Fullerton Ave., Chicago 14, Ill., U.S.A.
Hsu, T. C., Dr.—University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston 25, Texas, U.S.A.
Hubby, J. L., Dr.—University of Texas, Austin, Texas, U.S.A.
Huestis, R. P., Prof.—Dept. of Biology, University of Oregon, Eugene, Ore., U.S.A.
Huestis, R. P., Mrs.*—Dept. of Biology, University of Oregon, Eugene, Ore., U.S.A.
Hughes, R. D., Prof.—Medical College of Virginia, Richmond 19, Va., U.S.A.
Hughes, R. D., Mrs.*—Medical College of Virginia, Richmond 19, Va., U.S.A.
Hunt, E. E., Jr., Dr.—Peabody Museum, Harvard University, Cambridge, Mass., U.S.A.
Hunter, A. F., Dr.*—Charlottesville, Va., U.S.A.
Hunter, A. W. S., Dr.—Horticulture Division, Canada Dept. of Agriculture, Central Experimental Farm, Ottawa, Ont., Canada
Hunter, A. W. S., Mrs.*—Horticulture Division, Central Experimental Farm, Ottawa, Ont., Canada
Hunziker, J. H., Dr.—Instituto de Botanica, Ministerio de Agric., Buenos Aires, Argentina
Husfeld, B., Dr.†—Forschungs-Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Landaul Pfalz, Germany
Hutchinson, A. H., Prof.—University of British Columbia, Vancouver 8, B.C., Canada
Hutt, F. B., Prof.—Rice Hall, Cornell University, Ithaca, N.Y., U.S.A.
Hutt, F. B., Mrs.*—Rice Hall, Cornell University, Ithaca, N.Y., U.S.A.
Hyde, B. B., Dr.—University of Oklahoma, Norman, Oklahoma, U.S.A.
Hyun, S. K., Prof.—Institute of Forest Genetics, Suwon, Kyunggido, Korea

Ikeda, Y., Dr.—University of Tokyo, Institute of Applied Microbiology, Tokyo, Japan
Imai, M., Dr.—Kyoto University, Kyoto, Japan
Imai, M., Mrs.*—Kyoto University, Kyoto, Japan
Irwin, J. O., Dr.—Medical Research Council, Statistical Research Unit, London School of Hygiene, London W.C.1, England
Irwin, J. O., Mrs.*—London School of Hygiene, London W.C.1, England
Irwin, M. R., Prof.—Dept. of Genetics, University of Wisconsin, Madison 6, Wis., U.S.A.
Iseki, S., Dr.—Dept. of Legal Medicine, Gunma University, Maebashi, Gunma Pref., Japan
Ishikawa, T., Dr.—Gibbs Research Laboratories, Yale University, New Haven, Conn., U.S.A.
Ishitani, C., Dr.—Dept. of Biological Sciences, Stanford University, Stanford, Calif., U.S.A.
Ito, T., Dr.—Biophysical Lab., St. Paul's University, Tokyo, Japan
Ives, P. T., Dr.—Dept. of Biology, Amherst College, Amherst, Mass., U.S.A.

Jaap, R. G., Dr.—Ohio State University, Columbus, Ohio, U.S.A.
Jaap, R. G., Mrs.*—4435 Shield's Place, Columbus, Ohio, U.S.A.
Jackson, C. E., Dr.—Cayler-Nickel Clinic, Riverview Dr., Bluffton, Ind., U.S.A.
Jackson, C. E., Mrs.*—Riverview Dr., Bluffton, Ind., U.S.A. (Penny & Janice)
Jacob, F., Dr.—Institut Pasteur, 28 rue de Dr. Roux, Paris 15, Seine, France
Jahn, A. H., Miss—Biology Dept., Brookhaven National Laboratory, Upton, N.Y., U.S.A.
James, A. P., Dr.—Atomic Energy of Canada Ltd., Chalk River, Ont., Canada
Janick, J., Prof.,—Dept. of Horticulture, Purdue University, Lafayette, Ind., U.S.A.
Jay, G. E., Jr., Dr.—National Institute of Health, U.S. Public Health Service, Bethesda, Md., U.S.A.

Jaynes, R. A., Mr.*—Yale University, Connecticut Agricultural Experimental Station, P.O. Box 1106, New Haven, Conn., U.S.A.
Jenkins, B. C., Dr.—Division of Plant Science, University of Manitoba, Winnipeg, Man., Canada
Jenkins, B. C., Mrs.*—271 Maplewood Ave., Winnipeg, 13, Man., Canada
Jenkins, J. A., Prof.—Dept. of Genetics, 348 Hilgard Hall, University of California, Berkeley 4, Calif., U.S.A.
Jimenez, H., Dr.—Universidad Central, Faculdad de Agronomia, Maracay, Aragua, Venezuela
Johansen, E. M., Miss—University of Wisconsin, Madison, Wis., U.S.A.
Johansson, I., Dr.—Dept. of Animal Breeding & Genetics, Royal Agriculture College of Sweden, Artillerigaten 36, Uppsala, Sweden
Johnson, A. G., Mr.—University of Minnesota, St. Paul, Minn., U.S.A.
Johnson, A. G., Mrs.*—Rt. 1, Box 166, Excelsior, Minn., U.S.A. (Carol Ann, Ralph & Noel)
Johnson, A. G., Dr.—British National Vegetation Research Station, Wellesbourne, Warwick, England
Johnson, A. S., Dr.—Poultry Division, Central Experimental Farm, Ottawa, Ontario, Canada
Johnson, A. S., Mrs.*—Poultry Division, Central Experimental Farm, Ottawa, Ontario, Canada
Johnson, B. L., Dr.—University of California at Los Angeles, Los Angeles, Calif., U.S.A.
Johnson, L. P. V., Prof.—Dept. of Plant Science, University of Alberta, Edmonton, Alta., Canada
Johnston, O., Prof.—North Texas State College, Denton, Texas, U.S.A.
Johnston, J., Miss*—1801 W. Mulberry, Denton, Texas, U.S.A.
Jolicoeur, P., Dr.—Walker Museum, University of Chicago, Chicago 37, Ill., U.S.A.
Jones, D. F., Dr.—Connecticut Agriculture Experiment Station & Yale University, P.O. Box 1106, New Haven, Conn., U.S.A.
Jones, L. M., Mr.—513 Normal Road, DeKalb, Ill., U.S.A.
Josie, G. H., Dr.—Dept. of National Health & Welfare, Ottawa, Ontario, Canada
Jucci, C., Dr.—Centre of Genetics, National Council Research, Instituto Spallanzani, Università, Pavia, Italy
Judd, B. H., Dr.—University of Texas, Austin, Texas, U.S.A.
Jugenheimer, R. W., Prof.—University of Illinois, Urbana, Ill., U.S.A.
Juhasz, S. E., Dr.—Royal Edward Laurentian Hospital, 3674 St. Urbain St., Montreal, P.Q., Canada
Juhasz, S. E., Mrs.*—3674 St. Urbain St., Montreal, P.Q., Canada

Kafer-Boothroyd, E., Dr.—Dept. of Genetics, McGill University, Montreal, P.Q., Canada
Kalle, G., Mr.—University of Pennsylvania, Philadelphia, Pa., U.S.A.
Kallman, F. J., Prof.—Columbia University, 722 W. 168th St., New York 32, N.Y., U.S.A.
Kallman, F. J., Mrs.*—Columbia University, 722 W. 168th St., New York 32, N.Y., U.S.A.
Kalmus, H., Dr.—Galton Laboratory, University College, Gower St., London, W.C.1, England
Kalter, H., Dr.—Children's Hospital Research Foundation, Elland & Bethesda, Cincinnati 29, Ohio, U.S.A.
Kalter, H., Mrs.*—Children's Hospital Research Foundation, Elland & Bethesda, Cincinnati, Ohio, U.S.A., (Jules, Henry & John)
Kanehisa, T., Dr.—Kobe University, Kobe, Japan
Kaplan, A. S., Dr.—Division of Microbiology, Albert Einstein Medical Centre, York & Tabor Rds., Philadelphia 41, Pa., U.S.A.
Kaplan, A. S., Mrs.*—Division of Microbiology, Albert Einstein Medical Centre, York & Tabor Rds., Philadelphia 41, Pa., U.S.A.
Kaplan, B., Miss—Brookhaven National Laboratory, Upton, N.Y., U.S.A.
Kaplan, W. D., Dr.—City of Hope Medical Centre, Duarte, Calif., U.S.A.
Kappert, H. C. H., Dr.†—Institut für Vererbungs- und. Züchtungsforschung, Berlin-Dahlem, Albrecht Thaer-Weg 6, Germany
Kasatchenko, A. S., Dr.*—51 Prince Arthur St., W., Montreal, P.Q., Canada
Kashyap, T. S., Dr.—Kashibhawan, Kapurthala (Punjab), India
Kato, M., Dr.*†—Dept. of Physiology, Faculty of Medicine, Sakiyo-Kn, Kyoto, Japan
Kato, R., Dr.*—Children's Cancer Research Foundation, 35 Binney St., Boston 15, Mass., U.S.A.
Kaudewitz, F., Dr.†—Max-Planck Institut für Virusforschung, Melanchthanst, Tübingen, West Germany

Kaufmann, B., Dr.—Carnegie Institute of Washington, Cold Spring Harbor, N.Y., U.S.A.
Keeler, C., Prof.—Georgia State College for Women, Milledgeville, Ga., U.S.A.
Keep, E., Miss—East Malling Research Station, East Malling, nr. Maidstone, Kent, England
Keeshan, C. P., Sister*—College of St. Rose, Albany, N.Y., U.S.A.
Keim, W. F., Dr.—Dept. of Agronomy, Purdue University, Lafayette, Ind., U.S.A.
Keiter, F., Dr.—Gerichtsanthropologisches Laboratorium, Breitenfelderstrasse 62, Hamburg, West Germany
Keller, E. C., Jr. Mr.—State University of Pennsylvania, State College, Pa., U.S.A.
Kelner, A., Prof.—Brandeis University, Waltham, Mass., U.S.A.
Kelton, D., Miss—Hatch Laboratory, University of Massachusetts, Amherst, Mass., U.S.A.
Kemp, G. A., Mr.—Canada Experimental Farm, Lethbridge, Alta., Canada
Kemp, T., Prof.—Institute for Human Genetics, Tagensvej 14, Copenhagen N., Denmark
Kenworthy, W., Dr.—Arnold Biological Laboratory, Brown University, Providence, R.I., U.S.A.
Kenworthy, W., Mrs.*—Arnold Biological Laboratory, Brown University, Providence, R.I., U.S.A. (James & Paul)
Kenyon, W. A., Prof.—Hamline University, St. Paul 4, Minn., U.S.A.
Kenyon, W. A., Mrs.*—1650 Hewitt Ave., St. Paul 4, Minn., U.S.A.
Keosian, J., Prof.†—Rutgers University, 40 Rector St., Newark 2, N.J., U.S.A.
Kerber, E. R., Mr.—Cereal Breeding Laboratory, Box 322, University of Manitoba, Winnipeg, Man., Canada
Kerr, E. A., Dr.—Horticultural Experimental Station, Vineland, Ont., Canada
Khan, S. I., Dr.—Central Experimental Farm, Ottawa, Ont., Canada
Khitrinski, V., Dr.—All-Union Selection Genetical Institute, Oridiopolskoje Shosse 5, Odessa, U.S.S.R.
Khoo, U., Dr.—Connecticut Agriculture Experiment Station, New Haven, Conn., U.S.A.
Kidd, H. J., Dr.—Pioneer Hi-Bred Corn Company, P.O. Box 203, Wamego, Kan., U.S.A.
Kidwell, J. F., Dr.—University of Nevada, Reno, Nev., U.S.A.
Kihara, H., Dr.—National Institute of Genetics, Yata 1, 111, Mishima, Japan
Kikkawa, H., Prof.—Dept. of Genetics, Faculty of Medicine, Osaka University, 33 Joancho, Kitaku, Osaka, Japan
Kim, C., Dr.—152 Insadong, Changro-ku, Seoul, Korea
Kimball, R. F., Dr.—Biology Division, Oak Ridge National Laboratory, P.O. Box Y, Oak Ridge, Tenn., U.S.A.
Kincaid, C. M., Dr.†—110 McCord Hall, University of Tennessee, Knoxville, Tenn., U.S.A.
King, J. W. B. Dr.—Animal Breeding Research Organization, 6 South Oswald Rd., Edinburgh 9, Scotland
King, S., Dr.—Poultry Branch, Agriculture Research Service, U.S.D.A., Lafayette, Ind., U.S.A.
Kirk, H., Mr.*—University of Saskatchewan, Saskatoon, Sask., Canada
Kita, F., Dr.—University of Hokkaido, Hokkaido, Japan
Kitani, Y., Dr.—Kyoto University, Kyoto, Japan
Kitzmiller, J., Prof.—University of Illinois, Urbana, Ill., U.S.A.
Kloepfer, W., Dr.—Dept. of Anatomy, Tulane University, New Orleans, La., U.S.A.
Knight, R. J., Dr.†—Blandy Experimental Farm, Boyce, Va., U.S.A.
Knott, D. R., Dr.—Field Husbandry Dept., University of Saskatchewan, Saskatoon, Sask., Canada
Knowles, R. P., Dr.—Forage Crops Section, Canada Agriculture Research Lab., University Sub Post Office, Saskatoon, Sask., Canada
Kobayashi, T. Prof.—Toyama University, Toyama, Japan
Koch, G., Dr.—Institut für Humangenetik, Waldeyerstr. 27, Münster, Westfalen, Germany
Kodani, M., Dr.—College of Medicine, State University of Iowa, Iowa City, Iowa, U.S.A.
Kojima, K., Mr.—Institute of Statistics, North Carolina State College, Raleigh, N.C., U.S.A.
Koller, P. C., Dr.†—Royal Cancer Hospital, Chester Beatty Research Institute, Fulham Rd., London S.W.3, England
Kolmark, G., Dr.—Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Koo, F. K. S., Dr.—Dept. of Agronomy & Plant Genetics, University of Minnesota, St. Paul 1, Minn., U.S.A.
Koopmans, A., Dr.—Genetisch Institut R.U., Rijksstraatweg 76, Haren, Groningen, Netherlands
Koppel-Goodman, H. G., Dr.*—26 Sherman Ave., White Plains, N. Y., U.S.A.

Koref-Santibanez, S., Dr.—University of Chile, Av. Zanartu, 1042, Santiago, Chile
Kosin, I., Prof.—State College of Washington, Pullman, U.S.A.
Koske-Westphal, T., Dr.—Zoologisches Staat Institut, Bornplatz 5, Hamburg 13, West Germany
Kovalik, R., Miss—Institute of Parasitology, Macdonald College, Ste. Anne de Bellevue, P.Q., Canada
Kozinski, A., Dr.—Institute of Microbiology, Rutgers University, New Brunswick, N.J.. U.S.A.
Kriebel, H. B., Dr.*—Ohio Agriculture Experimental Station, Wooster, Ohio, U.S.A.
Krieg, D. R., Dr.—Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Krimbas, C., Dr.—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Krimbas, C., Mrs.*—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Kristjansson, F. K., Dr.—Central Experimental Farm, Ottawa, Ont., Canada
Kristjansson, F. K., Mrs.*—1031 Baseline Rd., Ottawa, Ont., Canada
Krueger, H. M., Prof.*—Oregon State College, Corvallis, Ore., U.S.A.
Krueger, R. G., Mrs.*—22 Country Club Way, Corvallis, Ore., U.S.A. (Farida Belle)
Kubryk, D., Dr.*—Dept. of National Health & Welfare, Ottawa, Ont., Canada
Kugelman, B., Miss—Pennsylvania State University, State College, Pa., U.S.A.
Kump, M., Dr.—Institute of Plant Breeding & Genetics, Faculty of Agriculture & Forestry, University of Zagreb, Yugoslavia
Kunicki-Goldfinger, W., Prof.—Dept. of Microbiology, The University, Wroclaw, Poland
Kunkel, H. A., Dr.—Universitäte-Frauenklinik, Stolberg Str. 7, Hamburg 21, Germany
Kupila, S., Dr.—Botanical Institute, Unionink 44, Helsinki, Finland
Kushner, D., Dr.—Forest Insect Laboratory, Sault Ste. Marie, Ont., Canada
Kushner, H. F., Prof.—Institute of Genetics, Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.
Kuspira, J., Mr.—University of Alberta, Edmonton, Alta., Canada
Kyle, W., Dr.—Poultry Breeding Projects, Purdue University, Lafayette, Ind., U.S.A.

LaChance, L. E., Mr.—Genetics Dept., North Carolina State College, Raleigh, N.C., U.S.A.
Lachance, P. W., Mr.—Institute of Parasitology, Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Lacour, L. F., Mr.—John Innes Horticultural Inst., Hertford, Herts, England
Lacy, A. M., Miss—Microbiology Dept., Yale University, New Haven, Conn., U.S.A.
Lagervall, M., Dr.—Dept. of Animal Husbandry, Iowa State College, Ames, Iowa, U.S.A.
Lagervall, M., Mrs.*—Dept. of Animal Husbandry, Iowa State College, Ames, Iowa, U.S.A. (Margareta)
Lake, B., Mrs.—American Genetic Association, Washington, D.C., U.S.A.
Laliberté, J., Mr.—Ferme Expérimentale, L'Assomption, P.Q., Canada
Lambert, J. W., Dr.*—Dept. of Agronomy, Institute of Agriculture, University of Minnesota, St. Paul, Minn., U.S.A.
Lamotte, M., Prof.—Ecole Nationale Supérieure, Paris, France
Lamotte, M., Mrs.*—rue Lhomond no. 24, Paris 5, France
Lamoureux, W. F., Dr.—Kimber Farms Inc., Niles, Calif., U.S.A.
Lamoureux, W. F., Mrs.*—P.O. Box 8, Niles, Calif., U.S.A.
Lampkin, G. H., Mr.—East African Veterinary Research Organization, Kikuyu, Kenya
Lampkin, G. H., Mrs.*—P.O. Box 32, Kikuyu, Kenya
Lamy, M., Prof.—Clinique de Génétique Médicale de la Faculté de Médecine, University of Paris, France
Landauer, W., Prof.—Genetics Dept., University of Connecticut, Storrs, Conn., U.S.A.
Landauer, W., Mrs.*—Genetics Dept., University of Connecticut, Storrs, Conn., U.S.A.
Langford, A. N., Prof.—Bishop's University, Lennoxville, P.Q., Canada
Langham, D., Dr.—The Sesamum Foundation, Maracay, Venezuela
Larivière, P., Dr.—3484 Laval, Montreal, P.Q., Canada
Larsen, C. S., Dr.—Royal Veterinary & Agriculture College, Horsholm, Denmark
Larson, R. I., Dr.—Science Service Laboratory, Lethbridge, Alta., Canada
Larter, E. N., Dr.—University of Saskatchewan, Saskatoon, Sask., Canada
Laskowski, W., Dr.—Max-Planck-Institut für vergleichende Errbiologie, Berlin-Dahlem, Germany
Lasley, E. L., Prof.—Animal Science Dept., University of Illinois, Urbana, Ill., U.S.A.
Latterell, R. L., Mr.*—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.

Laubscher, F. X., Dr.—Dept. of Genetics, University of Stellenbosch, Stellenbosch, South Africa
Laughnan, J. R., Prof.—Dept. of Botany, University of Illinois, Urbana, Ill., U.S.A.
Lauprecht, E., Prof.—Max-Planck-Institut für Tierzucht und Tierernahrung, Mecklenhorst, Germany
Laven, H., Dr.—Max-Planck-Institut für Biologie, Tübingen, Germany
Lawler, S. D., Dr.—Galton Laboratory, University College, Gower St., London, W.C.1, England
Lawrence, T., Dr.—Experimental Farm, Swift Current, Sask., Canada
Lawson, F. A., Miss*—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Lawson, N. C., Mr.—Dept. of Agronomy, Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Leak, L. V., Mr.—Dept. of Botany & Plant Pathology, Michigan State University, East Lansing, Mich., U.S.A.
Lebedev, M., Prof.—Pushkin Laboratory of Breeding Animals, Leningrad, U.S.S.R.
Leblond, C. P., Prof.*—Anatomy Dept., McGill University, Montreal, P.Q., Canada (Marie-Pascal)
Lecky, T. P., Dr.—Bodles Cattle Breeding Center, Old Harbour, Jamaica, B.W.I.
Lederberg, J., Prof.†—Dept. of Genetics, University of Wisconsin, Madison, Wis., U.S.A.
Ledingham, G. F., Prof.—Regina College, Regina, Sask., Canada
Lee, R., Prof.—Dept. of Genetics, Cornell University, Ithaca, N.Y., U.S.A.
Lee, W. R., Jr., Prof.—University of New Hampshire, Durham, N.H., U.S.A.
Lee, W.-J., Mr.—Dept. of Biology, College of Education, Seoul University, Seoul, Korea
Lefevre, G., Dr.—National Science Foundation, Washington, D.C., U.S.A.
Legates, J. E., Prof.—North Caroline State College, Raleigh, N.C., U.S.A.
Lejeune, J., Dr.—Chaire d'Hygiène et Clinique de la Premiere Enfancé (Faculté de Médecine), Paris, France
Lemay, J. P., Mr.—Ferme Expérimentale, Ste. Anne de la Pocatière, P.Q., Canada
Lenz, J. L., Mr.—Dept. of Zoology, University of Kansas, Lawrence, Kan., U.S.A.
Lerman, L. S., Dr.—Dept. of Biophysics, Medical Centre, University of Colorado, Denver, Col., U.S.A.
Lerner, I. M., Prof.—Poultry Dept., University of California, Berkeley 4, Calif., U.S.A.
Les, E., Mr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Lesher, S., Dr.*—Argonne National Laboratory, Lemont, Ill., U.S.A.
Lesins, K., Dr.—Dept. of Plant Science, University of Alberta, Edmonton, Alta., Canada
Leupold, U., Dr.—Institute of General Botany, University of Zurich, Zurich, Switzerland
Levene, H., Prof.—Dept. of Mathematical Statistics, Columbia University, New York 27, N.Y., U.S.A.
Levine, L., Dr.—Biology Dept., City College of New York, New York 31, N.Y., U.S.A.
Levine, L., Mrs.*—139 St. & Convent Ave., New York, N.Y., U.S.A. (Philip & Marilyn)
Levine, M., Dr.—Biology Dept., Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Levine, R. P., Dr.—The Biological Laboratories, Harvard University, Cambridge, Mass., U.S.A.
Levine, R. P., Mrs.*—The Biological Laboratories, Harvard University, Cambridge, Mass., U.S.A.
Levings III, C. S., Mr.*—Agronomy Dept., University of Illinois, Urbana, Ill., U.S.A.
Levins, R., Mr.—Columbia University, New York 27, N.Y., U.S.A.
Levinthal, C., Prof.—Massachusetts Institute of Technology, Cambridge, Mass., U.S.A.
Lewis, C. F., Prof.—Plant Industry Station, Beltsville, Md., U.S.A.
Lewis, E. B., Prof.—California Institute of Technology, Pasadena, Calif., U.S.A.
Lewis, H., Prof.—University of California at Los Angeles, Los Angeles, Calif., U.S.A.
Lewis, H., Prof.—Dept. of Biology, Massachusetts Institute of Technology, Cambridge, Mass., U.S.A.
Lewis, H., Mrs.*—Dept. of Biology, Massachusetts Institute of Technology, Cambridge, Mass., U.S.A., (David & Miriam)
Lewis, M., Miss—University of Manitoba, Winnipeg, Man., Canada
Lewontin, R. C., Prof.—North Carolina State College, Raleigh, N.C., U.S.A.
Li, C. C., Prof.—Graduate School of Public Health, University of Pittsburgh, Pittsburgh 13, Pa., U.S.A.
Li, C. C., Mrs.*—Graduate School of Public Health, Pittsburgh 13, Pa., U.S.A. (Carol & Steven)

Li, H. W., Dr.—Institute of Botany, Academia Sinica, c/o Taiwan Sugar Experiment Station, Taiwan, Free China
Lichtwardt, E., Mrs.†—University of Kansas, Lawrence, Kansas, U.S.A.
Lieb, M., Dr.—Brandeis University, Waltham, Mass., U.S.A.
Lima de Faria, A., Dr.—Institute of Genetics, University of Lund, Sweden
Lima de Faria, A., Mrs.*—University of Lund, Sweden
Lincoln, R. E., Dr.—1625 Shookstown Rd., Frederick, Md., U.S.A.
Lindegren, C. C., Dr.—Biological Research Laboratory, Southern Illinois University, Carbondale, Ill., U.S.A.
Lindegren, C. C., Mrs.*—Southern Illinois University, Carbondale, Ill., U.S.A.
Linden, D. B., Prof.—University of Florida, Gainesville, Fla., U.S.A.
Lindsley, D. L., Jr. Dr.—Oak Ridge National Laboratories, Oak Ridge, Tenn., U.S.A.
Lindsley, D. L., Jr. Mrs.*—Oak Ridge National Laboratories, Oak Ridge, Tenn., U.S.A. (Kathleen, Dale & Jennifer)
Loiselle, R., Dr.—Cereal Crops Division, Central Experimental Farms, Ottawa, Ont., Canada
Lonnquist, J. H., Dr.—University of Nebraska, Lincoln, Neb., U.S.A.
Loosli, R., Mr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Loosli, R., Mrs.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Lorz, A. P., Dr.—University of Florida, Gainesville, Fla., U.S.A.
Lorz, A. P., Mrs.*—R.R. #1, Micanopy, Fla., U.S.A.
Löve, A., Dr.—Institut Botanique, Université de Montréal, Montreal, P.Q., Canada
Löve, A., Mrs.*—4525 Kensington Ave., Montreal 28, P.Q., Canada (Goy & Loa)
Lowry, D. C., Mrs.—Poultry Husbandry Dept., University of California, Berkeley, Calif., U.S.A.
Lubinsky, G., Dr.*—Institute of Parasitology, Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Luce, W. M., Prof.—Dept. of Zoology, University of Illinois, Urbana, Ill., U.S.A.
Luce, W. M., Mrs.*—Dept. of Zoology, University of Illinois, Urbana, Ill., U.S.A.
Ludwig, W., Prof.—Zoologisches Institut, Universität Heidelberg, Heidelberg, Germany
Luers, H., Prof.—Institut für Genetik, 9 Rudeloffweg, Berlin, Germany
Luers, H., Mrs.*—Institut für Genetik, 9 Rudeloffweg, Berlin, Germany
Luria, S. E., Prof.—Bacteriology Dept., University of Illinois, Urbana, Ill., U.S.A.
Lush, J. L., Prof.—Animal Husbandry Dept., Iowa State College, Ames, Iowa, U.S.A.
Lush, J. L., Mrs.*—Animal Husbandry Dept., Iowa State College, Ames, Iowa, U.S.A.
Lynch, C. J., Dr.—Rockefeller Institute, York Ave. & 66th St., New York 2, N.Y.. U.S.A.
Lyon, M. F., Dr.—Radiobiological Research Unit, Harwell, Didcott, Berks., England

Ma, T. H., Mr.—Blandy Experimental Farm, Boyce, Va., U.S.A.
Maass, H., Dr.—Universität Frauenklinik, Hamburg 20, Germany
MacDonald, M. A., Dr.—Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Macdonald, M.D., Dr.—Science Service Laboratory, Canadian Dept. of Agriculture, Lethbridge, Alta., Canada
Mack, C., Miss*—Carnegie Institute of Washington, Cold Spring Harbor, N.Y., U.S.A.
MacKey, J. F., Dr.—Swedish Seed Association, Svalof, Sweden
Macklin, M. T., Dr.—Ohio State University, Rm. 122B Starling Loving, Columbus 10, Ohio, U.S.A.
MacLean, C. B., Mr.—St. Francois Xavier University, Antigonish, N.S., Canada
Madill, H. D., Mr.—Dept. of Plant Science, University of Alberta, Edmonton, Alta., Canada
Madill, H. D., Mrs.*—University of Alberta, Edmonton, Alta., Canada
Magee, W. E., Dr.—Upjohn Company, Kalamazoo, Mich., U.S.A.
Magni, G. E., Dr.—Instituto di Genetica, via S Epifanio 14, Pavia, Italy
Mainx, F., Prof.—Institut für Allgemeine Biologie, Wien 1X, Austria
Majumder, S. K., Mr.—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Majumder, S. K., Mrs.*—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Malogolowkin, C., Dr.—Faculdade Nacional de Filosofia, Universidade do Brazil, Rio de Janeiro, Brazil
Malcovati, P., Prof.—Ospedale Provinciale Maternita, via Palestro 10/20, Milano, Italy
Mange, A. P., Mr.—Dept. of Biology, Western Reserve University, Cleveland 6, Ohio, U.S.A.
Mangelsdorf, P. C., Prof.—Botanical Museum of Harvard, Cambridge, Mass., U.S.A.

Mangelsdorf, P. C., Mrs.*—Botanical Museum of Harvard, Cambridge, Mass., U.S.A.
Manna, G. K., Dr.—Dept. of Zoology, Bangabasi College, Calcutta 9, India
Manna, G. K., Mrs.*—Dept. of Zoology, Bangabasi College, Calcutta 9, India
Manunta, C., Prof.—Istituto di Zoologia, Universita di Sassari, Pavia, Italy
Marcano, L., Dr.—Centro de Investigaciones Agronomicas, Maracay, Venezuela
Marcou, D., Miss—Laboratoire de Génétique de la Faculté des Sciences, Paris 5, France
Marcovitch, H., Dr.—Laboratoire Pasteur, Institute du Radium, Paris, France
Maresca, L., Miss*—Cornell University, Ithica, N.Y., U.S.A.
Margolin, P., Dr.†—Brookhaven National Laboratories, Upton, L.I., N.Y., U.S.A.
Markarian, D., Dr.—Washington State College, Pullman, Wash., U.S.A.
Martin, G., Dr.—University of Washington, Seattle, Wash., U.S.A.
Martin, G. A., Mr.—Poultry Dept., North Carolina State College, Raleigh, N.C., U.S.A.
Martin, T. G., Prof.—Dairy Dept., Purdue University, Lafayette, Ind., U.S.A.
Martinoli, G., Prof.—Dept. of Botany, University of Pisa, Italy
Mather, K., Prof.—Dept. of Genetics, University of Birmingham, Edgbaston, Birmingham 15, England
Matsumoto, K., Dr.—Biological Laboratory, Osaka University of Liberal Arts and Education, Tennoji, Osaka, Japan
Matsushita, H., Dr.—Wakayama Medical School, Wakayama, Japan
Matsumura, S., Dr.—National Institute of Genetics, Yata 1, 111, Misima, Japan
Matzinger, D. F., Prof.—North Carolina State College, Raleigh, N.C., U.S.A.
Mauer, I., Mr.—Dept. of Medical Service, Medical Division Office, Squibb Institute for Medical Research, New Brunswick, N.J., U.S.A.
Mauer, I., Mrs.*—684 Main St., Metuchin, N.J., U.S.A. (Joanna)
Maw, A. J. C., Dr.—Pennsylvania State University, University Park, U.S.A.
Maw, W. A., Prof.*—Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Mayeda, K., Dr.—University of Utah, Salt Lake City 16, Utah, U.S.A.
Maynard-Smith, J., Mr.—Dept. of Zoology, University College, Gower St., London, England
Mazoti, L. B., Dr.—Instituto Fitatecnico de Santa Catalina, Buenos Aires, Argentina
McCann, L. P., Dr.*—U.S. Dept. of Agriculture, Bethesda, Md., U.S.A. (David & Kathleen)
McClung, M. R., Dr.—University of Rhode Island, Kingston, R.I., U.S.A.
McDonald, D., Dr.—Dept. of Biology, Dickinson College, Carlisle, Pa., U.S.A.
McDonald, D., Mrs.*—Dept. of Biology, Dickinson College, Carlisle, Pa., U.S.A.
McDonald, D. J., Dr.—St. John's College, Annapolis, Md., U.S.A.
McFadden, A. D., Dr.—Experimental Farm, Lacombe, Alta., Canada
McGibbon, W. H., Prof.†—University of Wisconsin, Madison, Wis., U.S.A.
McGinnis, R. C., Dr.—Cereal Breeding Laboratory, University of Manitoba, Winnipeg, Man., Canada
McGregor, W. G., Dr.—Cereal Crops Division, Experimental Farms Branch, Dept. of Agriculture, Ottawa, Ont., Canada
McIntosh, W. B., Dr.—Ohio State University, Columbus, Ohio, U.S.A.
McKay, G., Mr.*—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
McKenzie, H., Mr.—Cereal Breeding Laboratory, Experimental Farm, Lethbridge, Alta., Canada
McLaren, A., Dr.—Royal Veterinary College, London, England
McLennan, H. A., Mr.—Forage Crops Division, Central Experimental Farm, Canada Dept. of Agriculture, P.Q., Ottawa, Ont., Canada
McRae, D. L., Dr.—Montreal Neurological Institute, Montreal, P.Q., Canada
Mead, C. G., Dr.—Zoology Dept., Michigan State University, East Lansing, Mich., U.S.A.
Mead, C. G., Mrs.*—Zoology Dept., Michigan State University, East Lansing, Mich., U.S.A.
Mechelke, F., Dr.—Institut für Kulturpflanzenforschung, Gatersleben, D.D.R., Germany
Megnet, R., Dr.—Yale University, New Haven, Conn., U.S.A.
Megson, F. H., Dr.—American Cyanamid Co., Bound Brook, N.J., U.S.A.
Mehra, K. L., Mr.—Agronomy Dept., Oklahoma State University, Stillwater, Okla., U.S.A.
Mello-Sampayo, T. Y., Dr.—Estacao Agronomica Nacional, Oeiras, Portugal
Mello-Sampayo, T. Y., Mrs.*—Estacao Agronomica Nacional, Oeiras, Portugal
Melnyk, J. H., Dr.—University of Alberta, Edmonton, Alta,, Canada
Melnyk, J. H., Mrs.*—Ste. C 8760–96th Ave., Edmonton, Alta., Canada
Mendez, R. E. D., Dr.—La Molina Agricultural Experimental Station, La Molina, Peru

Menzel, M. Y., Dr.—Dept. of Biological Science, Florida State University, Tallahassee, Fla., U.S.A.
Mercier, E., Dr.—Ferme Expérimentale, Lennoxville, P.Q., Canada
Merrell, D. J., Dr.—Dept. of Zoology, University of Minnesota, Minneapolis, Minn., U.S.A.
Merritt, E. S., Dr.—Poultry Division, Central Experimental Farm, Canada Dept. of Agriculture, Experimental Farm Service, Ottawa, Ont., Canada
Merz, T., Dr.—The Johns Hopkins University, Baltimore, Md., U.S.A.
Merz, T., Mrs.*—The Johns Hopkins University, Baltimore, Md., U.S.A.
Metcalf, J. L., Prof.*—Wayne University, 81 Edson Ave., Detroit, Mich., U.S.A.
Metrakos, J. D., Dr.—Dept. of Genetics, McGill University, Montreal, P.Q., Canada
Metrakos, K., Dr.*—Dept. of Genetics, McGill University, Montreal, P.Q., Canada
Mettler, L. E., Dr.—Dept. of Biology, The Johns Hopkins University, Baltimore, Md., U.S.A.
Mettler, L. E., Mrs.*—The Johns Hopkins University, Baltimore, Md., U.S.A. (Tanya)
Meyer, H. E., Dr.—Dept. of Zoology, Indiana University, Bloomington, Ind., U.S.A.
Meyer, J. R., Dr.—U.S. Dept. of Agriculture & Mississippi Agriculture Experimental Station, Delta Branch Station, Stoneville, Miss., U.S.A.
Meyerhoffer, D. C., Mr.—Virginia Polytechnic Institute, Blacksburg, Va., U.S.A.
Michaelis, A., Dr.†—Institut für Kulturpflanzenforschung, Gatersleben, Germany
Michaelis, P., Dr.—Max-Planck-Institut für Züchtungsforschung, Köln-Vogelsang, Germany
Michie, D., Dr.†—Royal Veterinary College, London, N.W.1, England
Mickey, G. H., Prof.—Dept of Zoology, Louisiana State University, Baton Rouge, La., U.S.A.
Mickey, G. H., Mrs.*—Louisiana State University, Baton Rouge, La., U.S.A. (Don)
Mikaelsen, K., Dr.—Institute of Genetics & Plant Breeding, Vollebekk, Norway
Milani, R., Dr.—Istituto Zoologia, Universita Pavia, Pavia, Italy
Milani-Comparetti, M., Mr.—G. Mendel Institute, Piazza Galeno 5, Rome, Italy
Miles, O. K., Dr.*—c/o Dr. Mary Warters, 3568 Greenway Place, Shreveport, La., U.S.A.
Milkman, R. D., Mr.—Dept. of Zoology, University of Michigan, Ann Arbor, Mich., U.S.A.
Millar, J., Miss*—McGill University, Montreal, P.Q., Canada
Millar, K. M., Miss*—Hospital for Sick Children, 555 University Ave., Toronto, Ont., Canada
Miller, D. D., Dr.—University of Nebraska, Lincoln 8, Neb., U.S.A.
Miller, H. M., Dr.—The Rockefeller Foundation, New York, N.Y., U.S.A.
Miller, J. R., Mr.—Dept. of Genetics, McGill University, Montreal, P.Q., Canada
Miller, J. R., Mrs.*—Dept. of Genetics, McGill University, Montreal, P.Q., Canada (Joan & Elspeth)
Miller, J. W., Mr.*—Biology Dept., Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Miller, L., Mrs.*—c/o Prof. C. P. Oliver, 305 East 32nd St., Austin, Texas, U.S.A.
Miller, O. L., Mr.—University of Minnesota, St. Paul 8, Minn., U.S.A.
Miller, R. A., Dr.—University of Alberta, Edmonton, Alta., Canada
Miller, W., Dr.—School of Veterinary Medicine, University of California, Davis, Calif., U.S.A.
Minigawa, T., Dr.—Dept. of Zoology, University of Syracuse, Syracuse, N.Y., U.S.A.
Mishra, H. R., Mr.—University of Minnesota, St. Paul, Minn., U.S.A.
Miyake, T., Dr.—Carnegie Institute of Washington, Cold Spring Harbor, L.I., N.Y., U.S.A.
Mizushima, U., Dr.—Institute of Plant Breeding, Tohoku University, Sendai, Japan
Mohler, J. D., Dr.†—Dept. of Zoology, Oregon State College, Corvallis, Oregon, U.S.A.
Montalenti, G., Prof.—Istituto di Genetica, Universita di Napoli, Napoli, Italy
Monthey, L. G., Dr.—American Society of Agronomy, Madison, Wis., U.S.A.
Moore, R. J., Dr.—Botany & Plant Pathology Division, Canada Dept. of Agriculture, Science Service, Ottawa, Ont., Canada
Moorehead, P. S., Dr.—Dept. of Pathology, University of Pittsburgh, Pittsburgh 35, Pa., U.S.A.
Moree, R., Dr.—Zoology Dept. (WSC), State College of Washington, Pullman, Wash., U.S.A.
Morgan, D. T., Dr.—Dept. of Botany, University of Maryland, College Park, Md., U.S.A.
Morgan, W., Prof.—South Dakota State College, Brookings, S. D., U.S.A.
Moriwaki, D., Dr.—Dept. of Biology, Faculty of Science, Tokyo Metropolitan University, Tokyo, Japan
Morley, F. H. W., Dr.—Division of Plant Industry, C.S.I.R.O., Canberra, Australia
Morpurgo, G., Dr.—Istituto Superiore di Sanità, Roma, Italy
Morris, R., Dr.—Dept. of Agronomy, University of Nebraska, Lincoln 1, Neb., U.S.A.
Morrison, J. W., Dr.—Cereal Crops Division, Dept. of Agriculture, Central Experimental Farm, Ottawa, Ont., Canada

Morrison, J. H., Dr.—Michigan State University, East Lansing, Mich., U.S.A.
Mortimer, R. K., Dr.—University of California, Berkeley, Calif., U.S.A.
Morton, N. E., Dr.—Dept. of Medical Genetics, University of Wisconsin, Madison, Wis., U.S.A.
Moser, H., Dr.—Long Island Biological Association, Cold Spring Harbor, L.I., N.Y., U.S.A.
Moses, M. J., Dr.—Rockefeller Institute for Medical Research, New York, N.Y., U.S.A.
Mossige, J., Mrs.—Norsk. Hydro's Institute for Cancer Research, Radiumhospitalet, Norway
Mota, M., Dr.—Estação, Agronomica Nacional, Sacavem, Portugal (Manuel)
Motulsky, A. G., Dr.—University of Washington, Seattle, Wash., U.S.A.
Moutschen, J., Dr.—Institut de Morphologie Végétale, Laboratoire de Cytogénétique, University de Liege, Liege, Belgium
Moutschen-Dahmen, M., Dr.—Institut de Morphologie Végétale, Laboratoire de Cytogénétique, University de Liege, Liege, Belgium
Moxley, J., Mr.—Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Mukai, T., Dr.—Dept. of Biological Sciences, Purdue University, Lafayette, Ind., U.S.A.
Mukherjee, B., Dr.—University of Utah, Salt Lake City, Utah, U.S.A.
Muller, H. J., Prof.—Dept. of Zoology, Indiana University, Bloomington, Ind., U.S.A.
Muller, H. J., Mrs.*—Dept. of Zoology, Indiana University, Bloomington, Ind., U.S.A. (Helen)
Mulligan, G. A., Mr.—Botany & Plant Pathology Laboratory, Science Service, Ottawa, Ont., Canada
Mundkur, B., Dr.—c/o University of Puerto Rico, Rio Piedras, Puerto Rico
Mundkur, B., Mrs.*—c/o University of Puerto Rico, Rio Piedras, Puerto Rico
Munkres, K. D., Mr.—Kansas State College, Manhattan, Kan., U.S.A.
Munro, S., Dr.—Canada Dept. of Agriculture, Ottawa, Ont., Canada
Munshi, Z. A., Dr.—Agriculture College, Tandojam, West Pakistan, Pakistan
Müntzing, A., Prof.—Institute of Genetics, Lund, Sweden
Murakami, U., Prof.†—Research Institute of Environmental Medicine, Nagoya University, Furo-Eho, Chikusa Ku, Nagoya, Japan
Muramatsu, M., Mr.—304 Curtis Hall, University of Missouri, Columbia, Mo., U.S.A.
Murphy, R. P., Prof.—Dept. of Plant Breeding, Cornell University, Ithaca, N.Y., U.S.A.
Murray, B. E., Dr.—Dept. of Agriculture, Ottawa, Ont., Canada
Murray, H. R., Prof.—Dept. of Horticulture, Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Murray, H. R., Mrs.*—183 Acres Rd., Beaconsfield, P.Q., Canada
Murray, M. J., Dr.—A. H. Todd Company, P.O. Box 711, Kalamazoo, Mich., U.S.A.
Murty, B. R., Mr.—Central Tobacco Research Institute, Rojohmundry, India
Myers, B. J., Miss*—Institute of Parasitology, Macdonald College, McGill University, Ste. Anne de Bellevue, P.Q., Canada
Myers, W. M., Prof.—University of Minnesota, St. Paul, Minn., U.S.A.
Myers, W. M., Mrs.*—University of Minnesota, St. Paul, Minn., U.S.A. (Susan, Mary Jane & James)

Nachtsheim, H., Prof.—Max-Planck-Institut für vergl. Erbbiologie, Berlin-Dahlem, Germany
Nadeau, L., Mr.*—University of Montreal, Botanical Institute, 4101 Sherbrooke St. E., Montreal, P.Q., Canada
Nagata, T., Mr.*—742 John Jay Hall, Columbia University, New York, N.Y., U.S.A.
Nakao, Y., Dr.—Biological Institute, Kyoto Prefectural Medical College, Kyoto, Japan
Nakaya, R., Dr.—Dept. of Microbiology, Yale University, New Haven, Conn., U.S.A.
Naylor, J. M., Dr.—University of Saskatchewan, Saskatoon, Sask., Canada
Naylor, J. M., Mrs.*—202-11th St., Saskatoon, Sask., Canada (David & Margaret)
Neel, J. V., Prof.—Dept. of Human Genetics, University of Michigan, Ann Arbor, Mich., U.S.A.
Nelson, O. E., Prof.—Dept. of Botany & Plant Pathology, Purdue University, Lafayette, Ind., U.S.A.
Nemec, F., Mr.*—Quebec Seed Company, 3421 Grand Bldg., Montreal, Que., Canada
Newby, W. W., Dr.—Dept. of Genetics, University of Utah, Salt Lake City, Utah, U.S.A.
Newcombe, H. B., Dr.—Biology Branch, Atomic Energy of Canada Ltd., Chalk River, Ont., Canada
Niazi, I. A., Mr.—Dept. of Zoology, McGill University, Montreal, Que., Canada

Nicholson, H. H., Dr.—Range Experimental Farm, Box 340, Kamloops, B.C., Canada
Nicoletti, B., Dr.—Institute of Genetics, University of Naples, Naples, Italy
Nienstaedt, H., Dr.—Northern Institute of Forest Genetics, Lake State Forest Experimental Station, Rhinelander, Wis., U.S.A.
Nilan, R. A., Dr.—Dept. of Agronomy, State College of Washington, Pullman, Wash., U.S.A.
Nissen, Ø., Prof.—Agricultural College of Norway, Vollebekk, Norway
Nissen, Ø., Prof. Mrs.*—Agricultural College of Norway, Vollebekk, Norway
Norby, D., Dr.—Dept. of Biology, Virginia Polytechnic Institute, Blacksburg, Va., U.S.A.
Nordskog, A. W., Prof.—Poultry Dept., Iowa State College, Ames, Iowa, U.S.A.
Noronha-Wagner, M., Mr.—Estação Agronomica Nacional, Sacavem, Portugal
North, D. T., Mr.—University of Minnesota, St. Paul, Minn., U.S.A.
Norton, H. W., Jr., Mr.*—Holstein-Friesian Association of America, Brattleboro, Vt., U.S.A.
Nourse, D. V. C., Miss—Columbia University, New York City, N.Y., U.S.A.
Nuffer, M. G., Dr.—202 Curtis Hall, University of Missouri, Columbia, Mo., U.S.A.
Nujdin, N. Y., Prof.—Institute of Genetics, Academy of Sciences of U.S.S.R., Moscow 33, U.S.S.R.
Nursall, J. R., Dr.*—University of Alberta, Edmonton, Alta., Canada
Nussbaum, H. R., Mrs.—Dept. of Genetics, McGill University, Montreal, P.Q., Canada

Oakberg, E. F., Dr.—Oak Ridge National Laboratory, P.O. Box Y, Oak Ridge, Tenn., U.S.A.
Oberle, G. D., Prof.—Virginia Polytechnic Institute, Blacksburg, Va., U.S.A.
Oberle, G. D., Mrs.*— 902 Draper Rd., Blacksburg, Va., U.S.A. (William, Mary & Charles)
Ogilvie, I. S., Mr.*—Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q. Canada
Ohkura, K., Dr.—Dept. of Human Genetics, Tokyo Medical & Dental University, Tokyo, Japan
Ohnishi, E., Dr.—Biology Division, California Institute of Technology, Pasadena, Calif., U.S.A.
Oksala, T. A., Dr.—Institute of Genetics, University of Helsinki, Helsinki, Finland
Olah, L. V., Prof.—Duquesne University, Pittsburgh 19, Pa., U.S.A.
Olbrycht, T. M., Prof.—College of Agriculture, Kozuchowska 7, Wroclaw, Silezia, Poland
Olive, L. S., Prof.—Columbia University, New York 27, N.Y., U.S.A.
Oliver, C. P., Prof.—University of Texas, Austin, Texas, U.S.A.
Oliver, C. P., Mrs.*—305 East 32nd Street, Austin, Texas, U.S.A.
O'Malley, W., Mr.—Dept. of Agricultral Biochemistry, University of Arizona, Tuscon, Arizona, U.S.A.
Ono, F., Dr.—Metropolitan University, Tokyo, Japan
Oosthuizen, S. F., Prof.—Pretoria Hospital, Pretoria, Transvaal, Union of South Africa
Oppenheimer, C., Dr.†—Agricultural Research Station, Rehovat, Israel
Orr-Ewing, A., Dr.—Research Division, B.C. Forest Service, Victoria, B.C., Canada
Ortiz, E., Dr.—Laboratorio de Citogenetica, Serrano 113, Madrid 6, Spain
Osborne, R. H., Dr.—Sloan-Kettering Inst. (Div. of Preventive Medicine), New York, N.Y., U.S.A.
Oshima, C., Dr.—National Institute of Genetics, Yata 1,111, Misima, Japan
Oshima, C., Mrs.*—National Institute of Genetics, Yata 1,111, Misima, Japan
Oster, I. I., Dr.—202 Jordan Hall, Indiana University, Bloomington, Ind., U.S.A.
Ottolenghi, A., Mr.—3607 Chestnut St., Philadelphia, Pa., U.S.A.
Oullet, C., Mr.*—Ferme Expérimentale, L'Assomption, P.Q., Canada
Owen, R. D., Prof.—California Institute of Technology, Pasadena, Calif., U.S.A.
Owen, R. D., Mrs.*—California Institute of Technology, Pasadena, Calif., U.S.A.
Owen, S. P., Dr.—Upjohn Company, Kalamazoo, Mich., U.S.A.
Owen, S. P., Mrs.*—1431 East Cork St., Kalamazoo, Mich., U.S.A.

Paddock, E. F., Dr.—Dept. of Botany, Ohio State University, Columbus, Ohio, U.S.A. (Sarah & Frederic)
Pagé, E., Dr.*—University of Montreal, Montreal, P.Q., Canada
Paliwal, R. L., Dr.—Dept. of Botany, B.R. College, Agra University, Agra, U.P., India
Palm, J. E., Dr.—Wistar Institute, 36th St. and Spruce, Philadelphia 4, Pa., U.S.A.
Palmer, C. G., Dr.—8513 Compton Rd., Indianapolis, Ind., U.S.A.
Palmer, R., Dr.*—University of Indiana, Indianapolis, Ind., U.S.A. (Christoffer & Eve)

Pandey, K. K., Dr.—Botany & Zoology Bldg., Ohio State University, Columbus, Ohio, U.S.A.
Pandey, K. K., Mrs.*—Botany & Zoology Bldg., Ohio State University, Columbus, Ohio, U.S.A. (Ashoka)
Papazian, H., Dr.—University of Southern Illinois, Carbondale, Ill., U.S.A.
Parker, D. R., Dr.—Division of Life Sciences, University of California, Riverside, Calif., U.S.A.
Parsons, P. A., Dr.—Dept. of Genetics, Cambridge University, 44 Storey's Way, Cambridge, England
Parsons, P. A., Mrs.*—44 Storey's Way, Cambridge, England
Partanen, C. R., Dr.—Children's Cancer Research Foundation, Harvard Biological Laboratories, Cambridge 38, Mass., U.S.A.
Partridge, C., Dr.—Old Post Rd., Northford, Conn., U.S.A.
Paul, G. I., Dr.—Dept. of Genetics, McGill University, Montreal, P.Q., Canada
Paul, G. I., Mrs.*—6580 Monkland Ave., Montreal, P.Q., Canada (Janet, Timothy, Lois & Deborah)
Pauley, S. S., Prof.†—School of Forestry, University of Minnesota, St. Paul 1, Minn., U.S.A.
Pavan, C., Prof.—Dept. of Biology, University of Sao Paulo, C. Postal 8105, Sao Paulo, Brazil
Pavan, C., Mrs.*—C. Postal 8105, Sao Paulo, Brazil
Pavlovsky, O., Miss—Dept. of Zoology, Columbia University, New York, 27, N.Y., U.S.A.
Pearse, H. A., Dr.*—Wayne University, Franklin, Mich., U.S.A.
Pearson, H. M., Dr.*—58 Sunset Ave., Amherst, Mass., U.S.A.
Peets, D. L., Prof.—Sir George Williams' College, Drummond St., Montreal, P.Q., Canada
Peets, D. L., Mrs.*—3443 Melrose Avenue, Montreal, P.Q., Canada (Kenneth & Roberta)
Penrose, L. S., Prof.—University of London, London, W.C.1, England
Penrose, L. S., Mrs.*—1 Rodborough Rd., London, N.W.11, England (Shirley)
Perkins, D. D., Dr.—Dept. of Biological Sciences, Stanford University, Stanford, Calif., U.S.A.
Perry, T. O., Dr.—School of Forestry, University of Florida, Gainesville, Fla., U.S.A.
Perry, T. O., Mrs.*—School of Forestry, University of Florida, Gainesville, Fla., U.S.A.
Pershad, G.-D., Mr.—Iowa State College, Ames, Iowa, U.S.A. (Darshan)
Person, C., Dr.—Canada Dept. of Agriculture Research Laboratory, P.O. Box 322, University of Manitoba, Winnipeg, Man., Canada
Pert, J. H., Dr.—Connaught Research Laboratories, Toronto, Ont., Canada
Peters, H. F., Mr.—Range Experimental Farm, Manyberries, Alta., Canada
Peters, W., Dr.—Loyola University, 6525 Sheriden Rd., Chicago, Ill., U.S.A.
Peterson, P. A., Prof.—Genetics Dept., Iowa State College, Ames, Iowa, U.S.A.
Peterson, P. A., Mrs.*—Genetics Dept., Iowa State College, Ames, Iowa, U.S.A.
Peterson, R. F., Dr.—Cereal Breeding Laboratories, P.O. Box 322, University of Manitoba, Winnipeg, Man., Canada
Peterson, R. F., Mrs.*—Cereal Breeding Laboratories, P.O. Box 322, University of Manitoba, Winnipeg, Man., Canada
Petras, M. L., Mr.—Essex College, Assumption University of Windsor, Windsor, Ont., Canada
Philip, U., Dr.—Dept. of Zoology, King's College, Durham University, Newcastle-on-Tyne, England
Philips, J. N., Dr.—Bristol Laboratories, P.O. Box 657, Syracuse 1, N.Y., U.S.A.
Pickett, R. C., Dr—Dept of Agronomy, Purdue University, West Lafayette, Ind., U.S.A.
Pickup, T., Mr.—Macdonald College of McGill University, Ste. Anne de Bellevue, P.Q., Canada
Pipkin, S. B., Dr.—Howard University, Washington, D.C., U.S.A.
Piternick, L., Miss—1174 Cragmont Ave., Berkeley 8, Calif., U.S.A.
Pittenger, T. H., Dr.—Marquette University, Milwaukee, Wis., U.S.A.
Piza, S de, T., Prof.†—Escola Superior de Agric. "Luiz de Queiroz" da Université de Sao Paulo, Piracicaba, E. de Sao Paulo, Brazil
Planansky, K., Dr.—Veterans Administration Hospital, Canadaigua, N.Y., U.S.A.
Plessers, A. G., Dr.—Department of Agriculture, Ottawa, Ont., Canada
Plough, H. H., Prof.—Amherst College, Amherst, Mass., U.S.A.
Plough, H. H., Mrs.*—Middle St., R.F.D. 1, Amherst, Mass., U.S.A.
Poirier, R., Dr.*—Pioneer Feeds, 3455 Prudhomme. Montreal, P.Q., Canada
Pond, V., Miss—Brookhaven National Laboratory, Upton, N.Y., U.S.A.

Pons, J., Dr.—Instituto "Bernardino de Sahagún" de Antropologia y Etnalogia, Barcelona, Spain
Post, R., Dr.—Columbia University, New York 27, N.Y., U.S.A.
Poulson, D., F., Prof.—Osborne Zoological Laboratories, Yale University, New Haven, Conn., U.S.A.
Povilaitis, B., Dr.—Tobacco Substation, Delhi, Ont., Canada
Prakken, R., Prof.—Laboratory of Genetics, Wagenigen, The Netherlands
Prevost, G. G., Dr.—Cité du Cardinal Lemoine, Paris V, France
Prevosti, A., Dr.—Centro de Genetica Animal y Humana (C.S.I.C.), Barcelona, Spain
Priadcencu, A., Dr.†—Cabinetul Secretului Prim, Academia Republicii Populare Romine, Bucarest, Roumania
Price, S., Dr.—U.S. Dept. of Agriculture, Plant Industry Station, Beltsville, Maryland, U.S.A.
Pritchard, R. H., Dr.—Dept. of Genetics, University of Glasgow, Glasgow, Scotland
Puck, T. T., Prof.—Dept. of Biophysics, University of Colorado, Denver 20, Col., U.S.A.
Pugsley, A. T., Dr.—Agriculture Research Institute, Wagga, N.S.W., Australia
Purser, A. P., Mr.—Animal Breeding Research Organization, 6 South Oswald Rd., Edinburgh, Scotland

Quadling, C., Dr.—Bacteriology Division, Science Service, Carling Ave., Ottawa, Ont., Canada
Quintanilha, A., Prof.—Centro de Investigacao, Cientifica Algodoeira, Lisbon, Portugal

Rajhathy, T., Dr.—Cereal Crops Division, Central Experimental Farm, Ottawa, Ont., Canada
Ramel, C., Dr.—Institute of Genetics, University of Stockholm, Stockholm, Sweden
Ramirez, D., Miss*—University of Philippines, Laguna, The Philippines
Raper, J. R., Prof.—Biological Laboratories, Harvard University, Cambridge 38, Mass., U.S.A.
Rasmusen, B. A., Dr.—School of Veterinary Medicine, University of California, Davis, Calif., U.S.A.
Rasmuson, B., Dr.—Institute of Systematics and Genetics, Uppsala 7, Sweden
Rasmussen, I. E., Dr.—Istituto di Gènética, Pavia University, Pavia, Italy
Rasmussen, K., Dr.†—Central Experimental Farm, Ottawa, Ont., Canada
Rattenbury, J. A., Dr.—University of Auckland, Auckland, C.I., New Zealand
Rattenbury, J.A., Mrs.*—Box 2553, Auckland, C.I., New Zealand
Ratty, F. J., Dr.—Dept. of Zoology, San Diego State College, San Diego 15, Calif., U.S.A.
Rauch, H., Prof.—Ferndale Hall, University of Massachusetts, Amherst, Mass., U.S.A.
Rauch, H., Mrs.*—Ferndale Hall, University of Massachusetts, Amherst, Mass., U.S.A.
Ravin, A. W., Dr.—Dept. of Biology, University of Rochester, Rochester, N.Y., U.S.A.
Ray, C., Dr.—Dept. of Biology, Emory University, Atlanta, Ga., U.S.A.
Ray, D. T., Dr.—Howard University, Washington, D.C., U.S.A.
Ray, D. T., Mrs.*—415 College St., N.W., Washington, D.C., U.S.A. (Carolyn)
Reed, S. C., Prof.—Dight Institute, University of Minnesota, Minneapolis, Minn., U.S.A.
Reed, S. C., Mrs.*—Dight Institute, University of Minnesota, Minneapolis, Minn., U.S.A. (Catherine, William)
Reed, T. E., Dr.—Dept. of Human Genetics, University of Michigan, Ann Arbor, Mich., U.S.A.
Reeve, E. C. R., Dr.—Institute of Animal Genetics, West Mains Road, Edinburgh, Scotland
Reinbergs, E., Dr.—Field Husbandry Dept., Ontario Agricultural College, Guelph, Ont., Canada
Rempel, W. E., Dr.—University of Minnesota, St. Paul, Minn., U.S.A.
Rempel, W. E., Mrs.*—University of Minnesota, Staff House 16, Rosemount, Minn., U.S.A.
Rennie, J. C., Prof.*—Ontario Agricultural College, Guelph, Ont., Canada
Renshaw, J., Miss—Arnold Arboretum, Jamaica Plain, Mass., U.S.A.
Renwick, J. H., Dr.—Galton Laboratory, University College, Gower St., London, W.C.1, England
Rhoad, A. O., Dr.—King Ranch, Kingsville, Texas, U.S.A.
Rhoad, A. O., Mrs.*—King Ranch, Kingsville, Texas, U.S.A. (Dirk)
Ricciardi, I. R., Dr.—Oficina Sanitaria Panamericana W.H.O., O.M.S., Lima, Peru
Richardson, D., Prof.—Dept. of Botany & Plant Pathology, Purdue University, Lafayette, Ind., U.S.A.
Richter, A. A., Mr.—Genetics Dept., University of Wisconsin, Madison 6, Wis., U.S.A.

Rick, C. M., Dr.—Truck Crops Division, University of California, Davis, Calif., U.S.A.
Rick, C. M., Mrs.*—Truck Crops Division, University of California, Davis, Calif., U.S.A.
Rieger, R., Dr.†—Institut für Kulturpflanzenforschung, Gaterleben, D.D.R., Germany
Riley, H. P., Dr.—University of Kentucky, Lexington, Ky., U.S.A.
Riley, R., Dr.—Plant Breeding Institute, Trumpington, Cambridge, England
Rinke, E., Prof.*—University of Minnesota, St. Paul, Minn., U.S.A.
Ris, H., Prof.—Zoology Dept., University of Wisconsin, Madison 6, Wis., U.S.A.
Ritchie, G. R., Miss—Commonwealth Bureau of Animal Breeding & Genetics, King's Bldgs., West Mains Rd., Edinburgh 9, Scotland
Rizet, G., Prof.†—Faculté des Sciences, rue Pierre Curie, Paris V., France
Rizki, M. T. M., Dr.—Dept. of Biology, Reed College, Portland 2, Oregon, U.S.A.
Roberts, J. A. F., Dr.—Clinical Genetics Research Unit, Hospital for Sick Children, Great Ormond St., London, W.C.1, England
Roberts, J. L., Dr.*—University of Massachusetts, Amherst, Mass., U.S.A.
Roberts, M. R., Mrs.—Amherst College, Amherst, Mass., U.S.A.
Robertson, A., Dr.—Institute of Animal Genetics, West Mains Rd., Edinburgh 9, Scotland
Robertson, D. W., Dr.—Dept. of Agronomy, Colorado State University, Fort Collins, Col., U.S.A.
Robertson, F., Dr.—Institute of Animal Gentics, West Mains Rd., Edinburgh 9, Scotland
Robertson, J. G., Dr.—Canada Dept. of Agriculture, Science Service, Ottawa, Ont., Canada
Robinson, H. F., Prof.—Institute of Statistics, North Carolina State College, Raleigh, N.C., U.S.A.
Robson, E. B., Dr.—Galton Laboratory, University College, Gower St., London W.C.1, England
Rodriguez, A., Dr.—Blandy Experimental Farm, Boyce, Va., U.S.A.
Rohringer, K. E., Dr.—Saatzucht und Versuchsanstalt, Gleisdorf, Austria
Rollins, R. C., Prof.—Gray Herborium, Harvard University, Cambridge, Mass., U.S.A.
Rollins, W. C., Dr.—University of California, Davis, Calif., U.S.A.
Rollins, W. C., Mrs.*—University of California, Davis, Calif., U.S.A.
Roman, H., Prof.—University of Washington, Seattle, Wash., U.S.A.
Rommel, M., Mr.—Division of Plant Science, University of Manitoba, Winnipeg, Man., Canada
Roper, J. A., Dr.—Dept. of Genetics, University of Glasgow, Glasgow, Scotland
Roper, J. A., Mrs.*—University of Glasgow, Glasgow, Scotland (Susan)
Roscoe, M. V., Prof.—Botany Dept., McGill University, Montreal, Que., Canada
Rosin, S., Prof.—Zoological Institute, Berne, Switzerland
Ross, J. G., Prof.—South Dakota State College, Brookings, S.D., U.S.A.
Ross, J. G., Mrs.*—110 Sunnyview, Brookings, S.D., U.S.A. (Kenneth & George)
Rothenbuhler, W. C., Dr.—Dept. of Zoology and Entomology, Iowa State College, Ames, Iowa, U.S.A.
Rothenbuhler, W. C., Mrs.*—Dept. of Zoology and Entomology, Iowa State College, Ames, Iowa, U.S.A.
Rousseau, J., Dr.—National Museum of Canada, McLeod & Metcalfe Sts., Ottawa, Ont., Canada
Rubin, M., Dr.—Children's Hospital, University of Buffalo, Buffalo, N.Y., U.S.A.
Rubis, D. D., Dr.—University of Arizona, Tucson, Ariz., U.S.A.
Ruckenbauer, G., Miss—Ontario Veterinary College, Guelph, Ont., Canada
Rudenberg, L., Mrs.*—Botanical Society, 32 Ross Rd., Belmont, Mass., U.S.A.
Rudkin, G. T., Dr.—Institute for Cancer Research, Philadelphia, Pa., U.S.A.
Rudner, R., Miss*—Columbia University, New York City 27, N.Y., U.S.A.
Rudorf, W., Prof.—Max-Planck-Institut für Züchtungsforschung, Köln-Vogel, Germany
Rumke, C. L., Prof.—Maliesingel 27A, Utrecht, Netherlands
Runner, M., Dr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Ruskin, S. H., Dr.*—1306 David Broderick Tower, Detroit, Mich., U.S.A.
Russell, E. S., Dr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Russell, L. B., Dr.—Oak Ridge National Laboratories, Oak Ridge, Tenn., U.S.A. (David & Evelyn)
Russell, W. L., Dr.—Oak Ridge National Laboratories, Oak Ridge, Tenn., U.S.A.
Ryan, F. J., Prof.—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Ryan, F. J., Mrs.*—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.

Sachs, L., Dr.—Weizmann Institute of Science, Rehavoth, Israel
Sachs, L., Mrs.*—Weizmann Institute of Science, Rehovoth, Israel
Sackler, M., Mrs.—Institute for the Study of Human Variability, Columbia University, New York 27, N.Y., U.S.A.
Sadanga, K., Dr.†—Iowa State College, Ames, Iowa, U.S.A.
Sadykov, S., Dr.—Institute of Genetics and Plant Physiology, Lunacharskoe Shosse, Academgorodoc, Tashkent, U.S.S.R.
Saez, F. A., Prof.—Avenida Italia 3318, Montevideo, Uruguay
Sagawa, Y., Dr.—Dept. of Botany, University of Florida, Gainesville, Fla., U.S.A.
Sager, R., Dr.—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Saison, R., Miss—Ontario Veterinary College, Guelph, Ont., Canada
Saito, H., Dr.—Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan
Saito, H., Mrs.*—University of Tokyo, Tokyo, Japan (Jun)
Sampson, D. R., Dr.—Horticulture Division, Central Experimental Farm, Ottawa, Ont., Canada
Sanchez-Monge, E., Dr.—Centro de Mejora del Maiz, Instituto Nacional de Investigaciones Agronomicas, Madrid, Spain
Sand, S. A., Dr.—Connecticut Agricultural Experiment Station, New Haven 4, Conn., U.S.A.
Sang, J. H., Dr.—Agricultural Research Council, Poultry Research Centre, King's Bldgs., Edinburgh 9, Scotland
Sangster, H. L., Dr.—Rt. 1—Box 63, Boyds, Maryland, U.S.A.
Sangster, J., Miss*—McGill University, Montreal, P.Q., Canada
Santos, I. S., Dr.—University of the Philippines, Los Banos, Laguna, The Philippines
Sarvella, P., Dr.—Washington State College, Pullman, Wash., U.S.A.
Sato, G., Dr.—Brandeis University, Boston, Mass., U.S.A. (Denry, Susan & Nathan)
Sato, I., Dr.—Institute of Botany, Dept. of Science, Osaka University, Osaka, Japan
Saul, G. B., 2nd, Dr.—Dartmouth College, Norwich, Vt., U.S.A.
Savitsky, H., Dr.—Beet Sugar Development Foundation & U.S. Dept. of Agriculture, Salt Lake City, Utah, U.S.A.
Savitsky, V. F., Dr.—Beet Sugar Development Foundation & U.S. Dept. of Agriculture, Salt Lake City, Utah, U.S.A.
Sawin, P. B., Dr.—Box 847, Bar Harbor, Maine, U.S.A.
Sawin, P. B., Mrs.*—Box 847, Bar Harbor, Maine, U.S.A.
Sax, K., Prof.—Arnold Arboretum, Jamaica Plain, Mass., U.S.A.
Sax, K., Mrs.*—380 South St., Jamaica Plain, Mass., U.S.A.
Scanlan, G. M., Sister*—College of St. Rose, Albany, N.Y., U.S.A.
Schacht, L. E., Mr—University of Michigan, Ann Arbor, Mich., U.S.A.
Schalet, A., Dr.—Dept. of Biology, St. Louis University, St. Louis, Missouri, U.S.A.
Schalet, A., Mrs.*—1402 South Grand Blvd., St. Louis, Missouri, U.S.A.
Schecroun, J., Miss—Laboratoire de Génétique, Faculté des Sciences de Paris, Paris V, France
Scheinberg, S. 2nd, Mr.—U.S. Dept. of Agriculture, Animal Husbandry Division, Beltsville, Md., U.S.A.
Scherr, G. H., Dr.—Dept. of Bacteriology, College of Medicine, University of Illinois, Chicago, Ill., U.S.A.
Schick, R., Prof.—Institut für Pflanzenzüchtung, Deutsche Akademie der Landwirtschaftswissenschaften zu Berlin, Grose-Lusewitz, Rostock 11, Germany
Schick, R., Mrs.—Institut für Pflanzenzüchtung, Deutsche Akademie der Landwirtschaftswissenschaften zu Berlin, Grose-Lusewitz, Rostock 11, Germany
Schlager, G., Mr.—University of Kansas, Lawrence, Kan., U.S.A.
Schlehuber, A. M., Prof.—Agronomy Dept., Oklahoma State University, Stillwater, Okla., U.S.A.
Schlehuber, A. M., Mrs.*—2007 W. Arrowhead, Stillwater, Okla., U.S.A. (Rita & Tommy)
Schnell, F. W., Dr.—Scharnhorst, Kreis Neustadt—Ruebenberge, Germany
Scholz, F., Dr.†—Dept. of Zoology, Institut für Kulturpflanzenforschung, Gatersleben, Germany
Schrader, F., Prof.—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Schrader, S. H., Dr.—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Schreiber, G., Prof.—University of Minas Gerais, Belo Horitonte, Brazil
Schull, W. J., Dr.—University of Michigan, Ann Arbor, Mich., U.S.A.
Schultz, J., Dr.—Institute for Cancer Research, Fox Chase, Philadelphia 11, Pa., U.S.A.

Schultz-Larsen, J., Dr.—University Institute for Human Genetics, Copenhagen, Denmark
Schurin, M., Dr.—Biology Dept., Massachusetts Institute of Technology, Cambridge, Mass., U.S.A.
Schwartz, D., Dr.—Biology Dept. Oak Ridge National Laboratories, Oak Ridge, Tenn., U.S.A.
Schwartz, D., Mrs.*—Biology Dept., Oak Ridge National Laboratories, Oak Ridge, Tenn., U.S.A.
Schwartz, N., Mr.—Dept. of Zoology, Syracuse University, Syracuse 10, N.Y., U.S.A.
Schwartz, V., Dr.—Max-Planck-Institut für Biologie, Tübingen, Germany
Schwenk, A. C., Mr.—Dept. of Genetics, McGill University, Montreal, P.Q., Canada
Schwink, I., Dr.—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Scossiroli, R E., Dr.—Istituto di Genetica, Pavia, Italy
Scott, A., Dr.—Colby College, Waterville, Maine, U.S.A.
Scott, A., Mrs.*—Colby College, Waterville, Maine, U.S.A. (Janet)
Scott, J. P., Dr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Scott, J. P., Mrs.*—10 Hancock St., Bar Harbor, Maine, U.S.A. (Paul & David)
Scott, W. M., Dr.—Merk & Company, Valleyfield, P.Q., Canada
Sears, E. R., Dr.—108 Genetics Bldg., University of Missouri, Columbia, Mo., U.S.A.
Sears, L. M. S., Dr.—108 Genetics Bldg., University of Missouri, Columbia, Mo., U.S.A.
Seecof, R., Mr.†—Dept. of Zoology, University of Texas, Austin, Texas, U.S.A.
Seegers, W., Dr.—New York University, New York, N.Y., U.S.A.
Senn, H. A., Dr.—Botany & Plant Pathology Laboratory, Canada Dept. of Agriculture, Science Service Bldg., Ottawa, Ont., Canada
Senn, H. A., Mrs.*—Canada Dept. of Agriculture, Science Service Bldg., Ottawa, Ont., Canada
Sentz, J., Dr.—Dept. of Agronomy & Plant Genetics, University of Minnesota, St. Paul 1, Minn., U.S.A.
Sermonti, G., Prof.—Istituto Superiore di Sanità, Roma, Italy
Serra, A., Dr.†—Centro di Studi di Genetica Umana della Università di Milano, Corso Venezia 55, Milano, Italy
Serra, J. A., Prof.†—Institute of Zoology, Faculty of Science, Lisbon, Portugal
Shaklee, W. E., Dr.—5913-85th Place, Hyattsville, Md., U.S.A.
Shaklee, W. E., Mrs.*—5913-85th Place, Hyattsville, Md., U.S.A. (Ronald, Janelle & Lynda)
Shank, B., Dr.—South Dakota State College, Brookings, S.D., U.S.A.
Shapard, P. B., Miss—Zoology Dept., Smith College, Northampton, Mass., U.S.A.
Shapiro, N., Mr.—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Shapiro, N., Mrs.*—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A. (Jeffrey)
Sharma, A. K., Dr.—Cytogenetics Laboratory, Botany Dept., Calcutta University, Calcutta 19, West Bengal, India
Sharma, K. D., Mr.—Genetics Dept., McGill University, Montreal, P.Q., Canada (Manoj)
Shaw, D., Mr.—Dept. of Genetics, University of Wisconsin, Madison, Wis., U.S.A.
Shaw, R., Dr.—School of Medicine, University of Virginia, Charlottesville, Va., U.S.A.
Sheek, M. R., Dr.—Upjohn Company, Kalamazoo, Mich., U.S.A.
Sheely, W. E., Dr.—R.F.D. No. 2, P.O.B. No. 122, Springfield, Vt., U.S.A.
Sheppard, P. M., Dr.—University of Liverpool, Liverpool 3, England
Sheppard, P. M., Mrs.*—University of Liverpool, Liverpool 3, England
Sheppard, Mrs.*—University of Liverpool, Liverpool 3, England
Sheridan, W. J., Dr.*—605 Medical Arts Bldg., Chattanooga, Tenn., U.S.A.
Shiotani, I., Prof.*—Purdue University, Lafayette, Ind., U.S.A.
Shoffer, R., Prof.—University of Minnesota, St. Paul 1, Minn., U.S.A.
Shrivastava, S. K., Prof.*—University of Illinois, Chicago, Ill., U.S.A.
Shult, E. E., Mr.—Biological Research Laboratory, Southern Illinois University, Carbondale, Ill., U. S.A.
Shultz, F. T., Dr.—Route 1, Box 97B, Livermore, Calif., U.S.A.
Shumovich, W., Mr.—Canada Dept. of Agriculture, Experimental Farm, Harrow, Ont., Canada
Sidhu, B. S., Mr.—State Agricultural College, Cornell University, Ithaca, N.Y., U.S.A.
Siegel, A., Dr.—Botany Dept., University of California at Los Angeles, Los Angeles 21, Calif., U.S.A.
Siminovitch, L., Dr.—University of Toronto, Toronto, Ont., Canada

Simpson, N. E., Miss—University of Toronto, Toronto, Ont., Canada
Singleton, W. R., Prof.—Blandy Experimental Farm, University of Virginia, Charlottesville, Va., U.S.A.
Singleton, W. R., Mrs.*—1841 University Circle, Charlottesville, Va., U.S.A. (Thomas & Margaret)
Siniscalco, M., Dr.—Dept. of Genetics, University of Naples, Naples, Italy
Sinotô, Y., Dr.—International Christian University, Mitaka, Tokyo, Japan
Sinotô, Y., Mrs.*—1500 Osawa, Mitaka, Tokyo, Japan
Sirks, M. J., Prof.—Genetisch Institut R.U., Haren, Groningen, Netherlands
Sisken, J., Dr.—City of Hope Medical Centre, Duarte, Calif., U.S.A.
Skalinska, M., Prof.—University of Krakow & Polish Academy of Science, Krakow, Poland
Skorda, E., Miss*—Plant Breeding Institute, Thessaloniki, Greece
Skyring, A., Dr.—The Johns Hopkins Hospital, Baltimore, Md., U.S.A.
Skyring, A., Mrs.*—206 B Donnybrook Lane, Towson 4, Md., U.S.A. (Keren & Timothy)
Slatis, H., Dr.—Argonne National Laboratory, Lamont, Ill., U.S.A.
Slizynski, B. M., Dr.—Institute of Animal Genetics, West Mains Rd., Edinburgh, Scotland
Slizynska, H. Dr.—Institut of Animal Genetics, West Mains Rd., Edinburgh, Scotland
Slonimski, P., Dr.—Lamoratoire de Génétique Physiologique, Gif sur Yvette, Seine & Oise, France
Smirnov, A. I., Prof.—Embassy of the U.S.S.R., 285 Charlotte St., Ottawa, Ont., Canada
Smith, B. W., Dr.—Dept. of Genetics, North Carolina State College, Raleigh, N.C., U.S.A.
Smith, B. W., Mrs.*—Dept. of Genetics, North Carolina State College, Raleigh, N.C., U.S.A.
Smith, D. C., Prof.—Agronomy Dept., University of Wisconsin, Madison, Wis., U.S.A. (Richard)
Smith, E. C., Prof.—Acadia University, Wolfville, N.S., Canada
Smith, H. H., Dr.—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Smith, H. H., Mrs.*—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Smith, J., Dr.—Mt. Holyoke College, South Hadley, Mass., U.S.A.
Smith, J. B., Mr.—Botany Dept., University of Hawaii, Honolulu, Hawaii
Smith, S. G., Dr.—Forest Biology Division, Science Service, Canada Dept. of Agriculture, Forest Insect Laboratory, Sault Ste. Marie, Ont., Canada
Smith, W. K., Dr.—Moore Hall, University of Wisconsin, Madison, Wis., U.S.A.
Smith, W. K., Mrs.*—1409 Euclid Ave., Madison, Wis., U.S.A.
Smithies, O., Dr.—Connaught Medical Research Laboratories, University of Toronto, Toronto, Ont., Canada
Snell, G. D., Dr.—Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine, U.S.A.
Snyder, L. A., Dr.*—University of Minnesota, St. Paul, Minn., U.S.A.
Sobels, F. H., Dr.—Institute of Genetics, State University of Utrecht, Utrecht, Netherlands
Sokal, R. R., Dr.—Dept. of Entomology, University of Kansas, Lawrence, Kan., U.S.A.
Sokal, R. R., Mrs.*—University of Kansas, Lawrence, Kan., U.S.A. (David & Hannah)
Sokoloff, A., Dr.—The William H. Miner Agricultural Research Institute, Chazy, N.Y., U.S.A.
Somers, C., Miss—University of Texas, Austin, Texas, U.S.A.
Somsky, J., Mr.*—Hyline Poultry Farms, Dallas Centre, Iowa, U.S.A.
Spangelo, L. P. S., Mr.—Horticulture Division, Central Experimental Farm, Ottawa, Ont., Canada
Spärck, J. W., Dr.—Tuberculosis Immunization Research Centre, State Serum Institute, Copenhagen, Denmark
Sparrow, A. H., Dr.—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Sparrow, A. H., Mrs.*—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Speicher, B. R., Dr.—University of Maine, Orono, Maine, U.S.A.
Speicher, B. R., Mrs.*—12 University Place, Orono, Maine, U.S.A.
Spiess, E. B., Dr.—Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, Pa., U.S.A.
Spiess, E. B., Mrs.*—University of Pittsburgh, Pittsburgh, Pa., U.S.A. (Arthur & Bruce)
Spofford, J. B., Dr.—Dept. of Zoology, University of Chicago, Chicago 37, Ill., U.S.A.
Sprague, L. M., Dr.—Scripps Institute of Oceanography, La Jolla, Calif., U.S.A.
Srb, A. M., Prof.—Dept. of Plant Breeding, Cornell University, Ithaca, N.Y., U.S.A.
St. Lawrence, P., Dr.—Stanford University, Stanford, Calif., U.S.A.
Staats, J., Miss*—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.

Stadler, J., Dr.—Iowa State College, Ames, Iowa, U.S.A.
Stahl, W., Prof.—Institut für Tierzuchtforschung, Dummerstorf der Deutschen Akademie der Landwertschaftswissenschaften, Freilograthstrasse 14, Rostock, D.D.R., Germany
Stalker, H., Prof.—Zoology Dept., Washington University, St. Louis 5, Mo., U.S.A.
Stanton, W. R., Dr.—Ministry of Agriculture, Samaru, Zaria, Northern Nigeria
Stark, M., Mrs.*—307 E 17th St., New York, N.Y., U.S.A.
Stebbins, G. L., Prof.—Division of Genetics, University of California, Davis, Calif., U.S.A.
Steffensen, D., Dr.—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Steffensen, D., Mrs.*—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A. (Eric & Christine)
Stehr, G., Dr.—Forest Insect Laboratory, Sault Ste. Marie, Ont., Canada
Stein, K., Dr.—Zoology Dept., Mount Holyoke College, South Hadley, Mass., U.S.A.
Steinberg, A. G., Prof.—Dept. of Biology, Western Reserve University, Cleveland 6, Ohio, U.S.A.
Steinberg, A. G., Mrs.—Dept. of Biology, Western Reserve University, Cleveland 6, Ohio, U.S.A. (Arthur)
Steiner, E., Prof.—Botany Dept., University of Michigan, Ann Arbor, Mich., U.S.A.
Stern, C., Dr.—Zoology Dept., University of California, Berkeley, Calif., U.S.A.
Stern, C., Mrs.*—Zoology Dept., University of California, Berkeley, Calif., U.S.A.
Stern, H., Dr.—Dept. of Agriculture, Chemistry Division, Science Service, Ottawa, Ont., Canada
Stevenson, A. C., Dr.†—Director, Medical Research Council, Population Genetics Research Unit, Warneford Hospital, Oxford, England
Stevenson, H., Dr.—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Stevenson, R., Prof.†—East Tennessee State College Substation, Johnson City, Tenn., U.S.A.
Stevenson, R., Mrs.*†—East Tennessee State College Substation, Johnson City, Tenn., U.S.A.
Stewart, I. E., Mrs.*—American Institute of Biological Sciences, 2000 P. St., N.W., Washington 6, D.C., U.S.A.
Stich, H. F., Dr.—Dept. of Cancer Research, University of Saskatchewan, Saskatoon, Sask., Canada
Stiles, K. A., Dr.—Zoology Dept., Michigan State University, East Lansing, Mich., U.S.A.
Stimpfling, J. A., Dr.—Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.
Stimpfling, J. A., Mrs.*—Box 233, Bar Harbor, Maine, U.S.A. (Karen & Lynn)
Stinson, H., Dr.—Connecticut Agriculture Experimental Station, New Haven, Conn., U.S.A.
Stoletov, V., Prof.—Moscow Agricultural Academy, Moscow, U.S.S.R.
Stomps, T. J., Prof.†—Botanical Institute, Pl. Middenlaan 7, Amsterdam, Netherlands
Stone, D. E., Dr.—Tulane University, New Orleans, La., U.S.A.
Stone, D. E., Mrs.*—3481 Jean St., New Orleans, La., U.S.A. (Terry, Janne & Dianne)
Stone, L. E., Mr.—University of Nebraska, Lincoln, Neb., U.S.A.
Stone, W. H., Prof.—Dept. of Genetics, University of Wisconsin, Madison, Wis., U.S.A.
Stone, W. H., Mrs.*—Dept. of Genetics, University of Wisconsin, Madison, Wis., U.S.A.
Stone, W. S., Prof.†—Dept. of Zoology, University of Texas, Austin, Texas, U.S.A.
Stone, W. S., Mrs.*†—Dept. of Zoology, University of Texas, Austin, Texas, U.S.A.
Stormont C., Prof.—School of Veterinary Medicine, University of California, Davis, Calif., U.S.A.
Strandskov, H. H., Prof.—University of Chicago, Chicago, Ill., U.S.A.
Strauss, B. S., Dr.—Dept. of Zoology, Syracuse University, Syracuse 10, N.Y., U.S.A.
Stringam, E. W., Prof.—Division of Animal Science, University of Manitoba, Winnipeg, Man., Canada
Strömnaes, Oe., Dr.—Institute of Genetics, University of Oslo, Blindern, Norway
Strömnaes, Oe., Mrs.*—Institute of Genetics, University of Oslo, Blindern, Norway (Björn)
Strong, L. C., Dr.—Roswell Park Memorial Institute, Director, Biological Station, Springville, N.Y., U.S.A.
Strong, L. C., Mrs.*—196 North Buffalo St., Springville, N.Y., U.S.A. (Wilson)
Stroun, J., Dr.—Clinique de Médecine, Universitaire Genèva, Genèva, Switzerland
Stroun, M., Dr.—Institut Botanique, Universitaire Genèva, Genèva, Switzerland
Stubbe H., Dr.—Institute for the Study of Cultivated Plants, German Academy of Sciences, Gatersleben, Germany, D.D.R.
Sturtevant, A. H., Prof.—California Institute of Technology, Pasadena 4, Calif., U.S.A.
Sturtevant, A. H., Mrs.*—California Institute of Technology, Pasadena 4, Calif., U.S.A.

Stutz, H. C., Dr.—Dept. of Botany, Brigham Young University, Provo, Utah, U.S.A.
Stutz, H. C., Mrs.*—Brigham Young University, Provo, Utah, U.S.A.
Sueoka, N., Mr.†—California Institute of Technology, Pasadena, Calif., U.S.A.
Sulerud, R. L., Mr.—University of Nebraska, Lincoln 8, Neb., U.S.A.
Sullivan, R., Dr.—University of Kansas, Lawrence, Kan., U.S.A.
Sumption, L., Dr.—Animal Husbandry Dept., University of Nebraska, Lincoln 3, Neb., U.S.A.
Sumption, L., Mrs.*—Animal Husbandry Dept., University of Nebraska, Lincoln 3, Neb., U.S.A. (John & Gloria)
Sunder, J. H., Mr.—A. H. Gibson Laboratory, School of Medicine, University of Pittsburgh, Pittsburgh, Pa., U.S.A.
Suomalainen, E., Prof.—Institute of Genetics, University of Helsinki, Helsinki, Finland
Suomalainen, H. O. T., Prof.—Dept. of Experimental Zoology, University of Turku, Turku, Finland
Suskind, S., Dr.—McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Md., U.S.A.
Sutherland, T. M., Dr.—Colorado State University, Fort Collins, Col., U.S.A.
Sutton, H. E., Dr.—University of Michigan, Ann Arbor, Mich., U.S.A.
Suwanakitti, V., Dr.—Chulalongkorn University, Bangkok, Thailand
Suzuki, D. T., Mr.*—Amherst College, London, Ont., Canada
Svejda, F. J., Dr.—Dept. of Agriculture, Central Experimental Farm, Ottawa, Ont., Canada
Swan, H. S. D., Mr.—Pulp & Paper Research Inst., 3420 University St., Montreal, P.Q., Canada
Swann, M., Dr.—Texas University for Women, Denton, Texas, U.S.A.
Swanson, C. P., Prof.—The Johns Hopkins University, Baltimore 18, Md., U.S.A.
Swift, M. E., Dr.—Lederle Laboratories Division, American Cyanamid Co., Pearl River, N.Y., U.S.A.
Szende, K., Dr.†—Institute of Genetics, Budapest, Hungary

Tabata, M., Mr.—Dept. of Agronomy & Plant Genetics, University of Minnesota, St. Paul, Minn., U.S.A.
Takahashi, R., Dr.—Ohara Institute for Agricultural Biology, Okayama University, Okayama-ken, Japan
Takats, S. T., Dr.—Biology Dept., Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Tanaka, N., Dr.—Botanical Institute, Faculty of Science, University of Tokyo, Tokyo, Japan
Tanaka, Y., Dr.—National Institute of Genetics, Misima, Sizuoka-ken, Japan
Tashian, R., Dr.—University of Michigan, Ann Arbor, Mich., U.S.A.
Tavcar, A., Prof.—Institute of Plant-breeding and Genetics, Faculty of Agriculture, Zagreb, Yugoslavia
Taylor, J. H., Dr.—Botany Dept., Columbia University, New York 27, N.Y., U.S.A.
Taylor, L. W., Prof.—Dept. of Poultry Husbandry, University of California, Berkeley 4, Calif., U.S.A.
Taylor, L. W., Mrs.*—University of California, Berkeley, 4, Calif., U.S.A.
Taylor, St. C., Dr.—A.R.C. Animal Breeding Research Organization, 6 South Oswald Rd., Edinburgh, Scotland
Tazima, Y., Dr.—National Institute of Genetics, Yata 1,111, Misima, Sizuoka-ken, Japan
Tector, A., Miss—Dept. of Genetics, McGill University, Montreal, P.Q., Canada
Teodoreanu, N., Prof.†—Académie de la République Populaire Roumanie, Cazarmi No. 24, Bucarest, Roumanie
Therman, E., Dr.—Botanical Institute, Unionink 44, Helsinki, Finland
Thoday, J. M., Dr.—Dept. of Genetics, The University, Sheffield, England
Thoday, J. M., Mrs.*—Dept. of Genetics, The University, Sheffield, England
Thomas, C., Miss*—University of Wisconsin, Madison, Wis., U.S.A.
Thompson, J. S., Prof.—Anatomy Dept., University of Alberta, Edmonton, Alta., Canada
Thompson, M. W., Dr.—University of Alberta, Edmonton, Alta., Canada
Thompson, N. R., Dr.—Virginia Polytechnic Institute, Blacksburg, Va., U.S.A.
Thompson, W. P., President—University of Saskatchewan, Saskatoon, Sask., Canada
Throckmorton, L. H., Miss—University of Texas, Austin, Texas, U.S.A.
Ting, Y-c., Dr.†—Botanical Museum, Harvard University, Oxford St., Cambridge, Mass., U.S.A.
Tjio, J. H., Agr. Eng.—Estacion Experimental de Aula Dei, Zaragoza, Spain
Tjio, J. H., Mrs.*—Apartade 202, Zaragoza, Spain (Yu Hin)

Tokunaga, C., Dr.—Kobe College, Kobe, Japan
Toll, H. W., Mr.—730 Equitable Bldg., Denver 2, Col., U.S.A.
Toll, H. W., Mrs.*—777 Vine St., Denver, Col., U.S.A.
Tolle, A., Dr.—Tierarztliches Institut der Universität, Gottingen, Hildesheim, Germany
Tolle, A., Mrs.*—33 Merkelstrasse, Gottingen, Hildesheim, Germany
Touchberry, R. W., Dr.—Dept. of Dairy Science, University of Illinois, Urbana, Ill., U.S.A.
Towner, J. W., Dr.—College of Agriculture, Agriculture Experimental Station, University of California, 405 Hilgard Ave., Los Angeles 24, Calif., U.S.A.
Townsend, E. C., Mrs.*—1907 Lake Ave. S.W., Knoxville, Tenn., U.S.A.
Townsend, J. I., Dr.—Dept. of Zoology, University of Tennessee, Knoxville, Tenn., U.S.A.
Trasler, D. G., Dr.—Dept. of Genetics, McGill University, Montreal, P.Q., Canada
Trifunovic, V., Dr.—Agronomy Dept., College of Agriculture, University of Illinois, Urbana, Ill., U.S.A.
Tsitsin, N., Acad.†—The Main Botanical Garden of the U.S.S.R. Academy of Sciences, Moscow, U.S.S.R.
Tsuda, S., Dr.—Dept. of Anatomy, University of Washington, School of Medicine, Seattle, Wash., U.S.A.
Turpin, R., Prof.†—Avenue Victor Hugo 94, Paris, France
Twamley, B., Prof.—Ontario Agricultural College, Guelph, Ont., Canada

Uchida, I. A., Dr.—Hospital for Sick Children, Toronto 2, Ont., Canada
Uhl, C. H., Dr.—Botany Dept., Cornell University, Ithaca, N.Y., U.S.A.
Ulrich, H., Prof.—Zoologic Institut der Eidgenöss Techn. Hochschule, Zurich, Switzerland
Ulrich, H., Mrs.—Zoologic Institut der Eidgenöss Techn. Hochschule, Zurich, Switzerland
Ulrich, V., Prof.—Dept. of Agronomy & Genetics, West Virginia University, Morgantown, W. Va., U.S.A.
Unrau, J., Prof.—University of Alberta, Edmonton, Alta., Canada

Vaarama, A., Prof.†—Dept. of Botany, University of Turku, Turku, Finland
Vajrabhaya, T., Mr.—Cornell University, Ithaca, N.Y., U.S.A.
Valencia, J. I., Prof.—University of Buenos Aires & Atomic Energy Commission, Buenos Aires, Argentina
Valencia, R. M., Dr.*—University of Buenos Aires & Atomic Energy Commission, Buenos Aires, Argentina
Valentine, F. A., Dr.—College of Forestry, Syracuse University, Syracuse 10, N.Y., U.S.A.
Vallega, J. Dr.—Instituto de Fitotecnico, Castelar, Argentina
Van den Bosch, J., Dr.—State University of Leyden, & Netherlands Institute of Preventative Medicine, Hoge Ryndyk 17, Leyden, Netherlands
Van't Hof, J., Mr.—Michigan State University, East Lansing, Mich., U.S.A.
Van Valen, L., Mr.—Zoology Dept., Columbia University, New York 27, N.Y., U.S.A.
Vaughan, V. C., III, Prof.*—Medical College of Georgia, Augusta, Ga., U.S.A.
Vernon, E. H., Dr.†—Iberia Livestock Experimental Farm, Jeanerette, La., U.S.A.
Vernon, E. H., Mrs.*†—Iberia Livestock Experimental Farm, Jeanerette, La., U.S.A.
Vesely, J. A., Dr.*—Experimental Farm Service, Manyberries, Alta., Canada
Vestad, R., Dr.—Institute of Genetics & Plant Breeding, Agriculture College of Norway, Vollebekk, Norway
Vestad, R., Mrs.*—Inst. of Genetics & Plant Breeding, Agriculture College of Norway, Vollebekk, Norway. (Karen & Per)
Vetukhiv, M. O., Dr.—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Vezina, C., Dr.—Institut Agricole d'Oka (University of Montreal), LaTrappe, P.Q., Canada
Vickery, R. K., Jr., Prof.—Dept. of Genetics, University of Utah, Salt Lake City 12, Utah, U.S.A.
Vickery, R. K., Jr., Mrs.*—Dept. of Genetics, University of Utah, Salt Lake City 12, Utah, U.S.A. (David & Peter)
Vogel, F., Dr.—Max-Planck-Institut für vergl. Erbbiologie und Erbpathologie, Berlin, Germany
Vogel, H. J., Dr.—Institute of Microbiology, Rutgers, The State University, New Brunswick, N.J., U.S.A.
Vogel, H. J., Mrs.*—Institute of Microbiology, Rutgers, The State University, New Brunswick, N.J., U.S.A.

Vojtišková, M., Dr.—Czechoslovak Academy of Science, Institute of Biology, Prague 6, Czechoslovakia
Von Borstel, R. C., Mr.—Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Von Krosigk, C. M., Mr.—Heisdorf and Nelson Farms Inc., Kirkland, Wash., U.S.A.
Von Sengbusch, R., Dr.†—Max-Planck-Institut für Züchtungsforschung, Hamburg-Volksdorf, Germany
Von Wettstein, D., Dr.—Genetics Dept., Forest Research Institut, Stockholm 51, Sweden
Von Wettstein, D., Mrs.*—Genetics Dept., Forest Research Institut, Stockholm 51, Sweden

Waddington, C. H., Prof.—Institute of Animal Genetics, West Mains Rd., Edinburgh, Scotland
Wagner, R. F., Prof.—Dept. of Zoology, University of Texas, Austin, Texas, U.S.A.
Wagner, R. F., Mrs.*—Dept. of Zoology, University of Texas, Austin, Texas, U.S.A. (Philip, James & Ruth)
Wahrman, J., Dr.—Dept. of Zoology, The Hebrew University, Jerusalem, Israel
Wakonig, R., Dr.—Biology Dept., University of Saskatchewan, Saskatoon, Sask., Canada
Walker, D. I., Mr.—British West Indies Central Sugar Breeding Station, P.O. Box 505, Barbadoes, B.W.I.
Walker, E. M., Dr.*—University of Toronto, Toronto 5, Ont., Canada
Walker, N. F., Prof.—Dept. of Zoology, University of Toronto, Toronto 5, Ont., Canada
Walker, G. W. R., Dr.—Dept. of Plant Science, University of Alberta, Edmonton, Alta., Canada
Wallace, A. T., Dr.—Florida Agriculture Experimental Station, University of Floria, Gainesville, Fla., U.S.A.
Wallace, B., Dr.—The Biological Laboratory, Cold Spring Harbor, N.Y., U.S.A.
Walters, J. L., Prof.—University of California, Santa Barbara College, Goleta, Calif., U.S.A.
Walters, J. L., Mrs.*—University of California, Santa Barbara College, Goleta, Calif., U.S.A.
Walton, R. E., Mr.*—Iowa State College, Ames, Iowa, U.S.A.
Wang, C-W., Dr.—University of Florida, Gainesville, Fla., U.S.A.
Warburton, D., Mrs.—Dept. of Genetics, McGill University, Montreal, P.Q., Canada
Warburton, F. E., Mr.—Anatomy Dept., McGill University, Montreal, P.Q., Canada
Ward, C. L., Dr.—Dept. of Zoology, Duke University, Durham, N.C., U.S.A.
Warters, M., Dr.—Centenary College, Shreveport, La., U.S.A.
Warwick, B. L., Dr.†—Rt. 1, Box 82, McGregor, Texas, U.S.A.
Warwick, E. J., Dr.—Animal Husbandry Research Division, U.S.D.A., Beltsville, Md., U.S.A.
Warwick, E. J., Mrs.*—4212 Van Buren, University Park, Md., U.S.A. (James, Richard & Kenneth)
Wasserman, M., Dr.†—Dept. of Zoology, University of Texas, Austin, Texas, U.S.A.
Wasserman, M., Mrs.*†—Dept. of Zoology, University of Texas, Austin, Texas, U.S.A.
Watanabe, T., Dr.—Dept. of Zoology, Columbia University, New York 27, N.Y., U.S.A.
Watkins, P. W., Mr.*—University of Illinois, Urbana, Ill., U.S.A.
Watt, D. D., Dr.—Southeast Louisiana Hospital and Tulane University, Mandeville, La., U.S.A.
Weaver, G., Mr.—Dept. of Horticulture, Rutgers University, New Brunswick, N.J., U.S.A.
Webb, N. C., Jr., Dr.—University of Colorado, Denver, Col., U.S.A.
Webber, B. B., Mr.—Yale University, New Haven, Conn., U.S.A.
Webber, B. B., Mrs.*—Apt. 1-J, 985 Fairfield Ave., Bridgeport, Conn., U.S.A.
Weber, P., Miss—Smith Kline & French Laboratories, 1530 Spring Garden St., Philadelphia, Pa., U.S.A.
Weijer, J., Prof.—Dept. of Plant Science, University of Alberta, Edmonton, Alta., Canada
Weinstein, A., Dr.—Biological Laboratories, Harvard University, Cambridge 38, Mass., U.S.A.
Weir, J. A., Dr.—Zoology Dept., University of Kansas, Lawrence, Kan., U.S.A.
Weir, J. A., Mrs.*—2040 Louisiana, Lawrence, Kan., U.S.A. (Ann, Arnold)
Welch, R. M., Dr.†—Zoology Dept., University of Texas, Austin 12, Texas, U.S.A.
Wellhausen, E., Dr.—Rockefeller Foundation, Calle Londres #40, Mexico City, Mexico
Wellhausen, E., Mrs.*—Calle Londres #40, Mexico City, Mexico (Anita)
Wells, S. A., Dr.—Experimental Farm, Canada Dept. of Agriculture, Lethbridge, Alta., Canada
Welsh, J. N., Mr.—Cereal Breeding Laboratory, P.O. Box 322, University of Manitoba, Winnipeg, Man., Canada
Welsh, J. N., Mrs.*—Box 322, University of Manitoba, Winnipeg, Man., Canada (Susan)
Welshons, W. J., Mr.—Oak Ridge Memorial Laboratory, P.O. Box Y, Oak Ridge, Tenn., U.S.A.

Wenstrup, E. J., Rev. Dr.—St. Vincent's College, Latrobe, Pa., U.S.A.
Weresub, L. K., Dr.—Botany & Plant Pathology Laboratory, Science Service, Ottawa, Ont., Canada
Westergaard, M., Prof.—Institute of Genetics, University of Copenhagen, Copenhagen, Denmark
Westergaard, M., Mrs.*—Institute of Genetics, University of Copenhagen, Copenhagen, Denmark
Westergaard, K., Miss*—Institute of Genetics, University of Copenhagen, Copenhagen, Denmark
Wheat, J. D., Dr.—Kansas State College, Manhattan, Kan., U.S.A.
Wheeler, M. R., Dr.—The Genetics Foundation, University of Texas, Austin 12, Texas, U.S.A.
White, F. H., Mr.—Canada Dept. of Agriculture, (Tobacco Div.), 2072 Honeywell Ave., Ottawa, Ont., Canada
White, W. J., Prof.—Field Husbandry Dept., University of Saskatchewan, Saskatoon, Sask., Canada
Whitehouse, H. L. K., Dr.—Botany School, University of Cambridge, Cambridge, England
Whitehouse, R. N. H., Mr.—Plant Breeding Institute, Trumpington, Cambridge, England
Whiteside, A. G. O., Mr.—Canada Dept. of Agriculture, Central Experimental Farm, Ottawa, Ont., Canada
Whiting, P. W., Dr.—Dept. of Zoology, University of Pennsylvania, Philadelphia, Pa., U.S.A.
Whiting, P. W., Mrs.*—Dept. of Zoology, University of Pennsylvania, Philadelphia, Pa., U.S.A.
Whittinghill, M., Prof.—University of North Carolina, Chapel Hill, N.C., U.S.A.
Whittinghill, M.,Mrs.*—119 Maxwell Rd., Chapel Hill, N.C., U.S.A. (Alvin)
Wiener, A. S., Dr.—Serological Laboratory, 64 Rutland Rd., Brooklyn, N.Y., U.S.A. (Barbara & Phyllis)
Wilcox, A. N., Prof.—University of Minnesota, St. Paul, Minn., U.S.A.
Wilczynski, J. Z., Prof.—Biological Laboratory, Lebanese State University, Beirut, Lebanon
Wilkes, A., Dr.—Canada Dept. of Agriculture, Science Service Bldg., Ottawa, Ont., Canada
Wilkinson, C. F., Jr., Dr.—N.Y.U. Post-Graduate Medical School, 550 First Ave., New York 16, N.Y., U.S.A.
Williams, G., Miss*—108 Waters Hall, University of Missouri, Columbia, Mo., U.S.A.
Williams, L. F., Dr.—108 Waters Hall, University of Missouri, Columbia, Mo., U.S.A.
Williams, R. B., Jr., Dr.—Naval Medical Research Institute, Bethesda, Md., U.S.A.
Williams, R. B., Mrs.*—5818 Ipswich Rd., Bethesda, Md., U.S.A. (Richard)
Williams, W., Mr.—John Innes Horticultural Institute, Bayford, Hertford, Herts., England
Williamson, D. L., Mr.—University of Nebraska, Lincoln 8, Neb., U.S.A.
Willweber, E., Dr.—Biological Laboratory, Kyoto University, Kyoto, Japan
Wilson, D., Dr.—Long Ashton Research Station, Bristol, England
Wilson, F. D., Mrs.—University of Texas, Austin, Texas, U.S.A.
Wilson, G. B., Dr.—Dept. of Botany, Michigan State University, East Lansing, Mich., U.S.A.
Wilson, G. B., Mrs.*—Dept. of Botany, Michigan State University, East Lansing, Mich., U.S.A.
Wilson, K. S., Dr.—National Institute of Health, Bethesda 14, Md., U.S.A.
Wimber, D., Dr.—Biology Dept., Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Witkin, E. M., Dr.—College of Medicine, State University of New York, Brooklyn, N.Y., U.S.A.
Wolf, B. E., Dr.—Institut für Genetik der freien Universität, Berlin-Dahlem, Germany
Wolff, G. L., Dr.—Room 107, Bldg. T-18, National Cancer Institute, Bethesda 14, Md., U.S.A.
Wolff, G. L., Mrs.*—Room 107, Bldg. T-18, National Cancer Institute, Bethesda 14, Md., U.S.A.
Wolff, S., Dr.—Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.
Wolfson, A., Dr.*—Murray State College, Murray, Ky., U.S.A.
Wolsky, A., Prof.—Dept. of Biology, Fordham University, New York 58, N.Y., U.S.A.
Wood, D. R., Dr.—Colorado State University, Fort Collins, Col., U.S.A.
Wood, D. R., Mrs.*—Colorado State University, Fort Collins, Col., U.S.A. (Chester, Marilyn)
Woods, P., Dr.—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A.
Woods, P., Mrs.*—Brookhaven National Laboratory, Upton, L.I., N.Y., U.S.A. (Thomas, Karen)
Woodward, D., Mr.—Botany Dept., Gibbs Laboratory, Yale University, New Haven, Conn., U.S.A.
Woodward, V., Prof.—Agronomy Dept., Kansas State College, Manhattan, Kan., U.S.A.

Woolf, C. M., Dr.—Laboratory of Human Genetics, University of Utah, Salt Lake City, Utah, U.S.A.
Woolley, G. W., Prof.—Sloan-Kettering Institute, 444 E. 68th St., New York, N.Y., U.S.A.
Wright, J. E., Jr., Dr.—Dept. of Botany, Pennsylyania State University, University Park, Pa., U.S.A.
Wright, J. E., Jr., Mrs.*—Dept. of Botany, Pennsylvania State University, University Park, Pa., U.S.A. (William & Anne)
Wright, S., Prof.—University of Wisconsin, Madison, Wis., U.S.A.
Wright, S., Mrs.*—3905 Council Crest, Madison, Wis., U.S.A.
Wright, T. R. F., Mr.—Osborn Zoological Laboratory, Yale University, New Haven, Conn., U.S.A.
Wylie, A. P., Miss—Dept. of Botany, The University, Manchester 13, England

Yalvac, S., Dr.*—Dept. of Zoology, University of Nebraska, Lincoln 8, Neb., U.S.A.
Yamasaki, Y., Dr.—National Institute of Agricultural Sciences, Hiratsuka, Kanagawa Pref., Japan
Yamashita, K., Dr.—Biological Laboratories, Kyoto University, Kyoto, Japan
Yanase, T., Dr.—Dept. of Internal Medicine, University of Kyushu, Hakozaki-Machi, Sukuoka-Ken, Japan
Yang, S-J., Miss*—Dept. of Field Crops, North Carolina State College, Raleigh, N.C., U.S.A.
Yao, S-Y., Miss*—Dept. of Botany, Cornell University, Ithaca, N.Y., U.S.A.
Yarnell, S. H., Dr.—U.S. Southeastern Vegetable Breeding Laboratory, Charleston, S.C., U.S.A.
Yarnell, S. H., Mrs.*—P.O. Box 3177, St. Andrew's Branch, Charleston, S.C., U.S.A.
Yasuzumi, G., Dr.—Dept. of Anatomy, Nara Medical College, Kashihara, Japan
Yeatman, C. W., Mr.—Petawa Forest Experimental Station, Chalk River, Ont., Canada
Yeh, B. P-s., Miss—Louisiana State University, Baton Rouge, La., U.S.A.
Yerganian, G., Dr.—Harvard Medical School & Children's Cancer Research Foundation, 35 Binney St., Boston, Mass., U.S.A.
Yerganian, G., Mrs.*—Harvard Medical School & Children's Cancer Research Foundation, 35 Binney St., Boston, Mass., U.S.A.
Ying, K., Mr.*—Field Husbandry Dept., University of Saskatchewan, Saskatoon, Sask., Canada
Yosida, T. H., Dr.—Children's Cancer Research Foundation, 35 Binney St., Boston, Mass., U.S.A.
Yosida, T. H., Mrs.*—848 Beacon, Boston 15, Mass., U.S.A.
Yost, H. T., Dr.—Dept. of Biology, Amherst College, Amherst, Mass., U.S.A.
Yost, H. T., Mrs.*—41 Berkshire Terrace, Amherst, Mass., U.S.A.
Yu, C. K., Mr.—University of Ottawa, Ottawa, Ont., Canada
Yuasa, A., Prof.—Dept. of Biology, College of General Education, University of Tokyo, Tokyo, Japan
Yura, T., Dr.—Dept. of Microbiology, Yale University, New Haven, Conn., U.S.A.
Yura, T., Mrs.*—310 Cedar St., New Haven, Conn., U.S.A.

Zagaja, S., Mr.—Dept. of Horticulture, Rutgers University, New Brunswick, N.J., U.S.A.
Zalokar, M., Dr.—Dept. of Microbiology, Yale University, New Haven, Conn., U.S.A.
Zečević, L., Dr.—Biological Institute, Division of Plant Breeding & Genetics, Beograd, Yugoslavia
Zelle, M. R., Prof.—Dept. of Bacteriology, Stocking Hall, Cornell University, Ithaca, N.Y., U.S.A.
Zeller, J. H., Mr.—U.S. Dept. of Agriculture, Beltsville, Md., U.S.A.
Zeller, J. H., Mrs.*—U.S. Dept. of Agriculture, 2725–29th. St. N.W., Washington, D.C., U.S.A.
Zielinski, Q., Dr.—Oregon State College, Corvallis, Ore., U.S.A.
Zimm, G. G., Mrs.—1445 Clifton Park Rd., Schenectady, N.Y., U.S.A.
Zimmering, S., Dr.—Dept. of Biology, Trinity College, Hartford, Conn., U.S.A.
Zinder, N. D., Dr.—Rockefeller Institute for Medical Research, York Ave. & 66th St., New York 21, N.Y., U.S.A.
Zulueta, A. de, Prof.—Museo Nacional de Ciencias Naturales, Madrid 6, Spain

X INTERNATIONAL CONGRESS OF GENETICS

Proceedings of the

X INTERNATIONAL CONGRESS OF GENETICS

Comptes rendus du

Xe CONGRÈS INTERNATIONAL DE GÉNÉTIQUE

August 20–27, 1958

McGILL UNIVERSITY, MONTREAL

President

SEWALL WRIGHT

Vice-Presidents

C. BARIGOZZI
A. MÜNTZING
C. PAVAN
L. S. PENROSE
Y. SINOTO
A. STURTEVANT
W. P. THOMPSON

Volume II: Abstracts

University of Toronto Press, Toronto, Ontario, Canada

1958

Copyright ©, Canada, 1958
University of Toronto Press
Printed in Canada

Abbott, Ursula K., and Vigfus S. Asmundson. Further Studies with Scaleless—An Inherited Ectodermal Defect in the Fowl: II, Heat Production and Respiratory Quotient. *Poultry Husbandry Department, University of California, Davis, Calif., U.S.A.*

The inheritance and phenotypic expression of scaleless have been described by Abbott and Asmundson (1957). The original carrier New Hampshire stock was outcrossed in 1956 to 4 distinct breeds of chicken: Jungle Fowl, Cornish, Barred Plymouth Rock, and Single Comb White Leghorn. F_2 progenies from each cross, segregating for scaleless, were reared in the following year. An equal number of scaleless and normal progeny of similar breeding from each cross and from some relatively non-inbred New Hampshire stock were subjected either to hypothermic stress at one day of age (prolonged shock) to the LD50 point for scaleless or to timed periods of cold (intermittent shock). The former method gave most consistent results; accordingly, most of the results presented are based on this procedure. An equal number of scaleless and normal birds were maintained as controls. Survivors and controls were tested when 5–9 weeks old in specially designed chick respiration units, capable of housing 7 birds. In each trial 6 units containing an equal number of scaleless and normal birds of similar sex and breeding were used.

Heat production and respiratory quotients of scaleless and normal shocked and unshocked birds are presented. Scaleless and normal differed significantly while stressed and unstressed birds showed slight differences. Values for different crosses are given. The respiratory quotients of scaleless and normal birds also differed, scaleless being lower than normals. Some fasted-fed comparisons on the same birds indicated that heat production of scaleless birds tended to increase following a 24-hour fast in contrast to normals. With increasing weight the heat production of scaleless decreased while that of normals increased. Scaleless birds were more variable in heat production and in respiratory quotient than normal birds. The need for increased heat production to maintain normal body temperature undoubtedly accounts, at least in part, for the significantly lower egg production of scaleless than normal sisters. The higher heat output may also explain differences in the thyroid gland which is larger per unit of body weight in scaleless than in normal birds.

Adams, Elijah. *See* Schwinck, Ilse.

Adati, Syôzô. Cytogenetics of Japanese Wild Forage *Miscanthus* Species. *Faculty of Agriculture, Mie University, Tu, Mie-Ken, Japan.*

Miscanthus species are grown widely in the eastern parts of Asia, especially in Japan. *M. sinensis* is the most common species and has been used as forage grass all over Japan from ancient times though it is poor in nutritive substance. Its varieties, *M. sinensis* var. *condensatus* and *M. sacchariflorus,* which grow in humid

soil, proved to be rich in protein and fat and were also found to be palatable on the basis of chemical analyses and digestive experiments with rabbits. Intergeneric and interspecific hybridizations have been carried out. The plants are propagated by seed or vegetatively by division or even by cutting as, e.g., *M. sacchariflorus*. The genus *Miscanthus* includes 6 species in Japan.

The basic chromosome number in *Miscanthus* is 19 which is the highest basic chromosome number in Gramineae. I have found polyploid series of 2, 3, 4, 5 and 6x, and their karyotype analysis has been carried out.

Diploid species ($2n = 38$, *M. sinensis, M. floridulus* as *M. japonicus, M. tinctorius* and *M. oligostachyus*) have a very similar karyotype with one pair of long chromosones with intercalary trabant (*cs*–chromosome). Autotriploid M. *sinensis* var. *condensatus* ($2n = 57$) has 3 *cs*–chromosomes and many heteroploid plants have been raised when pollinated by diploid plants of which chromosome numbers ranged from 38 to 46. Tri-, tetra- and penta-ploid plants in *M. soccharifiorus* were found. The tetraploid plants ($2n = 76$) are common and have one pair of *cs*-chromosomes, therefore I suppose them to be allotetraploids. There are two kinds of triploid plants ($2n = 57$), one is sterile with 2 *cs*-chromosomes, while the other (*M. sacchariflorus* Bentham var. *brevibarbis* (Honda) Adati) is sterile with one *cs*-chromosome. Pentaploid plants ($2n = 95$, *M. sacchariflorus* form. *latifolius* Adati f. nov.) are very vigorous and have 4 *cs*-chromosomes. *M. intermedius* is autohexaploid ($2n = 114$) with 6 *cs*-chromosomes and grows in the northern parts of Japan.

AHUJA, Y. R. An Eye-Hair Human Mosaic. *Department of Genetics, University of California, Berkeley, Calif., U.S.A.*

This is a report of the rare case of a girl 20 years old, who shows partial bilateral asymmetry of eye and hair color. The color of her left iris is predominantly hazel with a brown pigment spot, while her right iris is predominantly blue with some yellow pigment spots. Most of the hair on her head is brown but there are two patches, one large and one small, of very light yellow hair on the right side. She does not appear to show asymmetry with regard to other characters. Her mother has dark brown eyes and hair. Her father has pale blue eyes and had blond hair in his youth but is bald now. Thus it is apparent that the eye and hair color predominant on the left side of the mosaic approximate those of her mother while the colors on the right side are more similar to those of her father.

In attempting to explain the causes of this mosaic condition it should be borne in mind that eye and hair color are not inherited in simple monogenic fashion. We may, however, assume that the dark or brown color of both the eyes and hair is partially dominant over blue eyes and blond hair respectively. Further, there is some evidence to the effect that degree of pigmentation of eye and hair is correlated.

The origin of bilateral asymmetry of the eye and hair color could be related, among others, to: (*a*) somatic mutations, (*b*) abnormal distribution of chromosomes during development, (*c*) somatic deletion, (*d*) somatic crossing-over. These mechanisms will be discussed.

The mosaic condition known as heterochromidia iridis and white forelock have both been reported to be inherited dominantly. However, it is not likely that the present mosaic condition will be transmitted.

Aksel, Rustem. *See* Johnson, L. P. V.

Alexander, Denton E. Genetic Induction of Autotetraploidy in Maize. *University of Illinois, Urbana, Ill., U.S.A.*

Maize plants homozygous for the recessive gene elongate (*el*) produce reduced and unreduced megaspores. Microsporogenesis is normal.

Agronomically useful inbred lines were outcrossed to *el/el* stocks, and the F_1's backcrossed to the parent inbred lines. The backcross progenies were self-pollinated, and the segregates subsequently crossed with established autotetraploid males. The *el/el* segregates bore triploid and tetraploid zygotes; the $\pm$ segregates produced triploid zygotes only.

Autotetraploids produced in this way were planted in isolation and permitted to interpollinate.

Open-pollinated diploid varieties are currently being converted to the tetraploid level using isolated crossing block techniques. The genetic diversity of the new tetraploid population should be almost as great as that which exists in the diploid population.

The basic object of the work in an idealistic sense is to transform a diploid species with all its genetic diversity to a tetraploid species.

Alexander, Mary L. The Relationship of Radiations and Environmental Changes in Oxygen Concentrations for Biological Damage in the Immature Germ Cells of *Drosophila virilis*. *Biology Department, University of Texas, M. D. Anderson Hospital, Houston, Tex., U.S.A.*

The post-meiotic cells of spermatogenesis of *Drosophila virilis* show differences in the amount of translocations and dominant lethals induced from radiation treatment. Meiotic and spermatogonial cells were different in that qualitative as well as quantitative differences in radiobiological damage have been found. The post-meiotic cells, which include mature non-motile sperm and several stages of spermatids, varied in sensitivity to X-rays, gamma rays and fast neutrons. With these radiations, the biological damage was consistently higher in spermatids than in mature sperm when oxygen was present at the time of treatment. The spermatid cells differ from mature sperm in that the same environmental concentration of oxygen, with radiation treatment, enhanced biological damage to a different extent. Oxygen concentrations over 20% (air) were not effective for increasing the damage in sperm cells; whereas, with spermatids, atmospheres of pure oxygen continued to increase the biological damage over the values obtained for air.

A slight but definite enhancement was obtained with oxygen with fast neutrons in the various types of immature germ cells. The amount of the increase in biological damage was not the same in all types of cells, indicating that the effectiveness of oxygen in the cells was not equal since all cells were treated in pure oxygen. Although oxygen enhances biological damage with neutrons, greater increases were evident with 200 Kv X-rays. The sensitivity of spermatids to oxygen was shown by increases in translocations with small changes in the environmental oxygen concentrations.

The lethal damage in the meiotic and spermatogonial cells was influenced by increases in oxygen concentrations with both X-ray and fast neutrons. With radiation

treatment, lethal damage in germ cells in the meiotic and spermatogonial stages may result from cell degeneration in the testes of the males or, secondly, from injury which survives these divisions and acts as a lethal in some later division.

Differences in the lethal damage induced in spermatids by gamma rays in pure oxygen were obtained by varying the dose rates of the radiation. However, mature sperm showed no differences in lethal damage with fast, slow or fractionated doses of gamma rays when treatments were made in an atmosphere of nitrogen. (Work supported by Contract No. AT-(40–1)-1828 between the United States Atomic Energy Commission and the University of Texas, M. D. Anderson Hospital.)

ALIKHANIAN, S. I., and T. S. ILJINA. Mutagenic Effect of Actinophage. *Antibiotics Research Institute, Moscow, U.S.S.R.*

In the plating of the phagolysate of *Actinomyces olivaceus*, N-6, obtained after its treatment with actinophage no. I in submerged conditions (in shake flasks) a high percentage of mutations is discovered. The frequency of morphological mutations reached 98%.

The typical colonies of strain N-6, without phage treatment, are dark grey and well sporulating. After phage treatment many mutants were discovered including white, light grey, light yellow, lemon yellow, film-like, and bacterial colony like mutants. Of great interest are biochemical mutations obtained under the action of actinophage. The frequency of appearance of biochemical mutations reaches 3.15% and greatly excels the ordinary frequency of biochemical mutations appearing under the action of other mutagenic factors, e.g., UV-rays.

In the course of studying the mutagenic action of a number of other actinophages belonging to the polyphage group it was discovered that some of the actinophages studied have their own "mutational spectrum" both in the frequency and in the character of mutations induced by them.

The influence of host culture (on which the phage multiplied itself) upon the "mutational spectrum" of the actinophage was studied. The mutations induced by two variants of one and the same phage were shown to be quite distinct in their character. For example, the actinophage grown on *Streptomyces griseus*, strain II (*A. globisporus* streptomycini after Krasilnikov), induces white, light yellow and lemon yellow mutations whereas the same phage grown on *Actinomyces olivaceus*, strain N-6, induces black and brown mutations. The influence of the host culture upon the mutagenic properties of actinophage gives us grounds to compare the phenomenon mentioned with the transduction phenomenon in bacteria.

ALIKHANIAN, S. I., and T. S. ILJINA. Recombinations in *Actinomyces Rimosus*. *Antibiotics Research Institute, Moscow, U.S.S.R.*

The discovery of recombinations in various species of bacteria on the one hand, and parasexual process in fungi on the other, allows one to assume that certain forms of sexual process exist in *Actinomycetes*, too, the latter being intermediate organisms between the two groups of organisms mentioned above. The breeding of hybrid forms of *Actinomycetes* is the more important as the majority of them are organisms producing antibiotics and are used in human practice.

By using the method of mixed growth of biochemical mutants, recombinant

forms of *Actinomyces rimosus*, a terramycin-producing actinomycete, are obtained experimentally. Recombinant forms both inside one and the same strains and between different strains of *Actinomyces rimosus* were shown to appear.

Various combinations of biochemical mutants are characterized by various frequency of appearance of recombinant forms. The frequency of recombinations within one given combination depends on the conditions of mixed growth of parent forms. Conditions permitting one to increase the frequency of recombinations up to 1,000 times are found. On the other hand, it is shown that in a liquid medium (both in shaking flasks and in stationary conditions) recombinations do not appear at all.

Appearance of several (2–3) types of prototroph forms, differing from one another both in morphological characters and in antibiotic activity, is discovered in some combinations of biochemical mutants. The proportion between different types of prototrophs appearing in a given combination changes with a change in conditions of parent forms growth.

Comparative studies of antibiotic activity of the recombinants, their parent forms (biochemical mutants), as well as the original strains (strains out of which the biochemical mutants were isolated), were carried out.

The antibiotic activity of the recombinants was shown to excel many times that of the parent forms. The activity of some of them reaches the activity of the original strains, or even excels it to a certain degree.

Essential differences between recombinants were discovered in the course of studying their stability. Some recombinants are absolutely stable and never segregate parent forms. Other recombinants in a number of generations always segregate biochemically deficient forms which are identical biochemically to one of the parent forms. Segregants corresponding to the other parent form were not discovered.

ALLEN, FRED H., Jr., PATRICIA A. CORCORAN, and LOUIS K. DIAMOND. Blood Groups of Eastern American Indians. *Blood Grouping Laboratory of Boston, Boston, Mass., U.S.A.*

Preliminary tests, done on 103 members of the Penobscot Tribe in Old Town, Maine, show blood-group frequencies quite similar to those that have been found in Indians of western North and South America, when allowance is made for some White admixture. In the preliminary tests, the following reagents were used:

anti-A, -B, -A_1, -H, -C, -c, -D, -E, -e, -C^w, -E^w, -M, -N, -M^g, -S, -Vw, -K, -Kp^a, Fy^a, -Jk^a, -P, -Le^a, -Le^b, Lu^a, -Wr^a, -Be^a, -Vel, and -Di^a.

As expected from the similarity to Western Indians of the other blood groups, the Diego (Di^a) factor was found in some of those tested. As expected from the known white admixture, some K-positive and Kp^a-positive individuals were found, indicating that linkage counts between Diego and Kell will probably be possible after further testing. Lu^a-positive was frequent enough to make it quite certain that further work will allow linkage counts between Diego and Lutheran. The greater frequency of Rh-negative than is found in groups previously studied aids in the identification of the rarer Rh genes. There are advantages in the racial admixture which appear to outweigh the possible disadvantages.

Work is in progress, and the lastest findings will be presented, particularly the linkage studies on the Diego blood groups.

ALLEN, GORDON. *See* PERLIN, SEYMOUR.

ALLISON, A. C. Metabolic Polymorphisms in Mammals. *National Institute for Medical Research, London, England.*

Recent studies have brought to light a number of genetically controlled differences in the proteins taken from different animals of the same species. These are of interest for three main reasons: they provide information about the way in which gene products can differ; they are responsible for some powerful selective effects that have interesting consequences in population genetics; and since the alleles controlling the characters are common in populations, they provide a number of marker loci for linkage studies.

These broad groups of metabolic polymorphisms can be distinguished. In one group each allele controls the formation of a distinct product and in the heterozygote both products are present. This is found, for example, in most of the haemoglobin types in man and other mammals, and in the lactoglobulin types in cattle. (Some of the human haemoglobin types differ by only a single amino-acid residue in each molecule.) Modifier genes are known to control the proportions of the two products in heterozygotes, in the extreme case suppressing one product altogether and thereby bringing about dominance modification. In the second group the two alleles produce distinct products, but the products in heterozygotes are intermediate. This seems to be true, for example, of some blood group genes and the human haptoglobins. Incidentally, it seems difficult to reconcile this finding with the template hypothesis of protein synthesis in its original form. Again, modifier genes are known which alter the products in the heterozygotes so that they are indistinguishable from those in one of the homozygotes, thereby bringing about dominance modification. In the third group the alleles control the formation of the same product but produce different amounts of it. This is true, for example, of the enzymes atropine esterase in rabbits and catalase in dog erythrocytes. Intermediate amounts of the product occur in heterozygotes, and this can also be enhanced or suppressed by modifiers until the amount of the product corresponds to that in one of the homozygotes. This is again an example of dominance modification. Other cases are on record where the primary biochemical differences are not yet known, e.g., the genetically controlled differences in sodium and potassium concentration in sheep erythrocytes.

ALTENBURG, EDGAR. *See* ALTENBURG, L. S.

ALTENBURG, L. S., and EDGAR ALTENBURG. The Induction of Visible Mutations at Selected Loci by Alkylating Agents. *Rice Institute, Houston, Texas, U.S.A.*

Using Muller's "Maxy" stock heterozygous for 13 recessive visibles in the X-chromosome, a preliminary study of the production of visible mutations at these loci by phenylalanine mustard and di-methyl myleran has been undertaken. A 10^{-2} M suspension of each agent was injected intra-abdominally into young males, and

their F_1 daughters were examined for visibles of all kinds. The lethal rate was obtained for comparison. Among 24,000 daughters of 257 mustard-treated males, 3 heritable visible mutations (*f*, *g*, and *w*) were found, and in addition there were 5 visibles associated with lethals, giving an average of 1 visible in 3,000 F_1 females. In the myleran-treated series from 142 treated males no heritable visibles were produced, but there were 7 visibles associated with lethals among 17,000 daughters, or a rate of 1 visible in 3,400 females. Among the controls 2 heritable visibles (*f*, *g*) were found in 45,000, or 1 in 22,500 females. The lethal mutation rate from the mustard series was $1.2 \pm 0.2\%$, and for the myleran series it was $2.0 \pm 0.3\%$.

All F_1 females were examined for mosaics for Maxy or other visible traits. In each of the two series of treated males an average of 1 mosaic for a Maxy trait in about 400 F_1 females was found. That many of these mosaics were gynanders seems likely although not many showed sex gynandromorphism. A rate of only 1 mosaic in 1,700 F_1 females was found in the control series. In the treated series only about one-sixth of the mosaics had heritable Maxy traits (a smaller proportion in the controls). A ratio of 1 complete Maxy visible to about 40 lethals was found in the treated series. If heritable traits in the mosaics are added to the complete visibles, the mutation rate for Maxy visibles in the mustard series becomes 1 in 1,200 F_1 females and in the myleran series 1 in 900 F_1 females; or a ratio of less than 1 Maxy visible to 10 lethals. (This work has been supported by a grant received for work of Edgar Altenburg and associates from the National Institute of Health, United States Public Health Service.)

ANDERS, FRITZ. On Sex Determination of *Gammarus pulex* L. *Zoologisches Institut der Universität des Saarlandes, Saarbrücken, Germany.*

Former investigations have shown that some autosomatic alleles of *Gammarus pulex* L., which cause red or reddish brown pigmentation of the integument, may cause a relative male-determining effect too, and that some combinations of these alleles may influence sex sufficiently to produce all or at least a majority of males (Anders, Vererbungsl. *88*: 291–332). In recent investigations it has been shown that the sex of *G. pulex* is determined by a large number of relative sex-determiners, which are distributed more or less equally over all the autosomes. Heterochromosomes were found neither in cytologic nor in genetic studies. Environmental factors have no perceptible influence on the sex-ratio.

ANDERSEN, A. C., and FRED T. SULTZ. A Developmental Cataract in the Dog. *School of Veterinary Medicine, University of California, Davis, Calif., U.S.A.*

Out of 1,129 purebred beagles observed 1 male showed complete bilateral cataract when 5 months of age. Matings with 5 normal dams produced 19 offspring with complete bilateral cataract, 2 with partial bilateral cataract, 1 nearly normal, and 3 normals. Puppies were sacrificed for gross histological study between 41 and 60 days of age. In all 3 outstanding defects were observed: microphthalmia, retinal folds, and lens opacity. This form of cataract seemed best explained as a defect in the development of the secondary lens fibers apparently resulting from incomplete

lens capsule continuity. Neither sex linkage nor an autosomal recessive was indicated. On the assumption of a dominant mutation, the probability of this large a deviation (21:4) in either direction from the expected ratio is 0.0009.

ANDERSON, RUTH S. *See* HUESTIS, R. R.

ARMSTRONG, J. M., and M. HATTERSLEY-SMITH. Polyspory in an Amphidiploid of *Triticum-Agropyron* Hybrids. *Forage Crops Division, Central Experimental Farm, Ottawa, Canada.*

An amphidiploid line in the F_9 generation tracing to the cross *Triticum turgidum* × *Agropyron intermedium* was found to contain plants which at meiosis showed a spindle abnormality. In plants carrying the abnormal gene over 50% of the microsporocytes contained five to eight microspores instead of the normal four. The occurrence of microspores with two nuclei was also common and was more prevalent in tetrads than in hexads to octads. Meiosis was found to be regular up to metaphase II. At this time a secondary spindle was formed and the chromosomes divided and moved to the poles of primary or secondary spindles. The number of chromosomes on secondary spindles ranged from three to one-half the complement of 32–35. After telophase II wall formation was usually initiated enclosing the resulting nuclei; where walls failed to form microspores were two-nucleate. The result of this abnormal gene was to bring about almost complete sterility in the affected plants.

ARNASON, J. T. *See* WAKONIG, RESA.

ARNOLD, C. G. *See* SCHWEMMLE, JULIUS, *et al.*

AR-RUSHDI, ABBAS H. The Polygenic Basis of Sex-Ratio in *Tigriopus*. *University College of Arts and Sciences, Baghdad, Iraq, and Scripps Institution of Oceanography, University of California, U.S.A.*

In the tide-pool copepod *Tigriopus*, extensive data derived from individual broodsacs corroborated previous reports on natural populations revealing a very striking deviation from a 1:1 sex-ratio. Preponderance of males being the observed condition in the majority of families.

Starting with a "randomized" parental population with 70% males, between-family selection was carried out for high and low-female lines. Against a calculated inbreeding coefficient of 0.7460, it has been possible to maintain lines in which the original sex-ratio was shifted to as high as 80% and as low as 0.5% females. Crosses among high-female lines and between high and low-female lines were also made. Inasmuch as mortality has been relatively low, and at that independent of sex-ratio, and in view of the absence of cytologically detectable chromosomal mechanisms for sex determination, data obtained indicate that in *Tigriopus* sex

is determined by a system of polygenes and that the heritability of the trait appears to be relatively high.

With the genetic mechanism of sex-determination hitherto revealed, and with the drastic fluctuations in population size observed in nature, it is concluded that drift to extreme sex ratios should be of frequent occurrence; that under the austere conditions often prevailing in the tide-pools, populations with high female ratio should be at a selective disadvantage considering, under the circumstances, that the female is the "more expensive" sex to maintain.

ASMUNDSON, VIGFUS S. *See* ABBOTT, URSULA K.

ASTAUROV, B. L. Artificial Parthenogenesis and Polyploidy in the Silkworm *Bombyx mori* L. *Severtzow Institute of Animal Morphology of Academy of Science of USSR, Moscow, U.S.S.R.*

By means of heat activation of silkworm unfertilized eggs it is possible to obtain up to 82% of complete parthenogenetic development and, of these parthenogenetic offspring, 100% are females. As in the natural thelytokys parthenogenesis, accompanied as a rule by polyploidy, the thermal artificial parthenogenesis facilitates the appearance and further preservation of polyploidy. Primary occurrence of polyploidy takes place in the course of parthenogenetic cleavage. When polyploidization occurs in the germ track cells, the parthenogenetic females produce large tetraploid eggs as well as normal diploid ones. The unfertilized $4n$-oocytes in their turn may be stimulated to parthenogenesis which leads to the appearance of the $4n$-females and later on of the corresponding parthenoclones. By crossing ♀ $4n$ × ♂ $2n$ the triploids of both sexes are easily produced. Owing to the aneuploidy of their gametes the triploids are quite sterile whether intercrossed or mated with normal diploids. But in the heat-activated unfertilized $3n$-oocytes, as in the $2n$ and the $4n$, the maturation proceeds ameiotically, the reduction division is suppressed and only the equation division remains. This opens the possibility of reproducing the triploids parthenogenetically, obtaining $3n$-clones and propagating them indefinitely by means of heat activation. Strangely enough some of the parthenogenetic $3n$-females when crossed to $2n$-males unlike their $3n$-mothers of bisexual origin are not absolutely sterile but give a small percentage of viable offspring of both sexes (approximately 1% on the average). The explanation is simple: whereas in diploid heat parthenogenesis sporadic polyploidization results in the appearance of $4n$-oocytes, in triploidy it leads to the appearance of $6n$-oocytes. When fertilized, the hexaploid oocytes maturate quite regularly and a $3n$-female pronucleus arises and after its union with the haploid sperm nucleus, a tetraploid zygote results. Previously the $4n$-males occurred in *Bombyx* as an extreme rarity. Now crossing of a parthenogenetic $3n$-female to the $2n$-male is the easiest way of obtaining $4n$-silkworms of both sexes in any desired number. Unfortunately the $4n$-males are sterile and a sexually propagating $4n$-race has not yet been obtained. Owing to the elaboration of the reliable method, which permits the induction of ameiotic parthenogenesis in *B. mori*, the production and investigation of experimental polyploids in this insect are more accessible than in any other animal.

AXFORD, S. J. *See* NEWCOMBE, H. B., *et al.*

BAILEY, CATHERINE H., and L. FREDRIC HOUGH. Inheritance of Season of Ripening in Certain Peach Progenies. *Rutgers, The State University of New Jersey, New Brunswick, N.J., U.S.A.*

The mode of inheritance of season of ripening in peaches has been studied using crosses between Jerseyland and five early-ripening varieties or selections including Prairie Daybreak, and between Prairie Daybreak and another early ripening selection.

There are six loci which act in a cumulative way to shorten the time required for fruit to ripen. All the parents studied are homozygous for the contributing genes at four of these loci. Specific modifiers have been postulated for five of these loci. There are three loci with dominant genes which shorten the development period by two or more weeks. Another locus shortens the development period by two weeks when it is in the recessive condition. Dominance, epistasis, duplicate factors and modifiers can account for most of the variation in the ripening season in these progenies. In one progeny some of the variation in the time of ripening resulted from segregation of contributing factors.

Jerseyland and Prairie Daybreak, the latest ripening parents used, are homozygous for five pairs of contributing factors. They are both heterozygous at another locus which shortens the development period by two weeks when it is in the recessive condition. Different modifiers are present in each. All of the five earlier ripening selections and varieties contain the dominant gene B_2 plus various modifiers. Four of these are homozygous for the same five pairs of contributing genes present in Jerseyland and Prairie Daybreak. The selection 7039 contains the dominant D, four pairs of contributing genes common to the other six parents and one other contributing gene. The Prairie Daybreak $\times$ 7039 cross was the only one producing seedlings ripening earlier than Mayflower.

BAJER, ANDRZEJ, and JADWIGA BAJER-MOLÉ. Mitosis in Living Cells—a Film: Normal, β-irradiated and Colchicine Mitosis. *Laboratory of Plant Physiology, Jagellonian University, Cracow, Poland.*

The endosperm of many species is suitable for studies of mitosis *in vitro*. The cells, without cellulose cell walls, can be flattened by surface tension. In such cells the length of the same chromosomes was measured from prophase to telophase and their volumes calculated. The length decreases throughout the whole of mitosis and the volume increases until the metaphase is reached and decreases during the anaphase. Observations on the course of mitosis indicate the following. The first sign of the spindle is the clear structureless zone which appears around the nucleus and when it reaches maximal dimensions the nuclear membrane breaks. This clear zone is the cytoplasmic constituent of the spindle. Formation of the clear zone is probably initiated by substances migrating from the nucleus.

The moment of nuclear membrane dissolution may be exactly estimated; it is followed by the sudden aggregation of the chromosomes in the central tighter mass (mitotic contraction stage). After this stage co-operation between kinetochores and the spindle takes place and the moment at which this begins (kinetochore stretching) may be seen in some cells.

During prometaphase the kinetochores do not always move to the equator by the shortest path and may move towards the pole and return. Acentric fragments are eliminated at the poles. Some chromosome arms execute peculiar movements (abrupt bending movements) which are absent in colchicine mitosis.

During the anaphase the changes inside the pushing body "Stemmkörper" cause the whole half-spindles to move apart. In the ana-telophase, during the formation of the phragmoplast, all particles and chromosome fragments are eliminated at the poles and the whole nuclei are pushed apart, giving the impression of a second anaphase during the early telophase.

BAJER-MOLÉ, JADWIGA. *See* BAJER, ANDRZEJ.

BAKER, HERBERT G. Observations on Heterostyly in Some Species of Tropical Plants. *Botany Department, University of California, Berkeley, Calif., U.S.A.*

The Rubiaceae, which provides important constituents of forest and savannah-vegetation in all parts of the tropics, is also celebrated as containing a larger number of heterostyled species than is yet known in any other family. In the tribe Psychotrieae, West African species of *Psychotria* and *Uragoga* are heterostylous and largely self-incompatible. In *Psychotria warneckei* K. Schum. and K. Krause, however, in addition to normally heterostylous plants, "cross-over" long-styled plants which are self-compatible have become established. This move towards secondary monomorphism may be correlated with the relatively unattractive flowers of this species and its weedy behaviour.

In the tribe Mussaendeae of the same family, at least two species of *Mussaenda* are found to be heterostylous (without the usual differences in pollen-size between long- and short-styled plants), but other species are shown to be functionally dioecious. The probable derivation of dioecism from heterostyly in this plant does not necessarily support the Darwinian belief that heterostyly is usually a stage on the path from hermaphroditism to dioecism, but is a special case deriving from the difficulty of transferring pollen from deep seated anthers to short styles in species with congested corolla-tubes.

In the Oxalidaceae, the trimorphic outbreeding system has been studied in the genera *Oxalis, Biophytum* and *Averrhoa.* Because of the greater complexity of this system compared with the dimorphic system, the number of ways in which it can break down to provide monomorphic, self-compatible populations is correspondingly greater. Illustrations of these are given. The correlation between monomorphism and weediness, on one hand, and establishment after apparently long-distance dispersal, on the other, are stressed for *Oxalis* and *Biophytum* and a brief comparison is made with conditions in the equally tristylous monocotyledonous family Pontederiaceae.

BAKER, WILLIAM K. and JANICE B. SPOFFORD. Heterochromatic Control of Variegation in *Drosophila melanogaster. Department of Zoology, University of Chicago, Chicago, Ill., U.S.A.*

It is well known in *Drosophila melanogaster* that extra Y-chromosomes suppress the extent of mutant areas in eyes which are variegated because of position effects at

the white locus. This phenomenon allows one to determine experimentally if the Y heterochromatin can be subdivided into functional genetic units. Seventeen different Y fragments were tested in three genotypes of coisogenic flies carrying a duplication of the w^+ locus inserted into the centromeric heterochromatin of chromosome 3L. This rearrangement produces variegated eyes if a *w* allele is carried in the X-chromosome. The three genotypes tested were: X/Y^F males, $X{\cdot}X/Y^F$ attached-X females, and $Y^SX{\cdot}Y^L$ attached-X-Y males. Both visual and chromatographic measurements were made on the extent of pigmentation and on the amount of ultraviolet fluorescing pteridines, respectively, present in the 51 different genotypes being compared. The evidence seems conclusive that Y heterochromatin contains discrete functional units. For example, cytological study of the Y fragments indicates that not only do fragments of the same cytological length have different potencies in suppressing variegation, but that certain large two-armed fragments are very ineffectual in modifying the size of the mutant area in variegated eyes. Quantitative determinations of the amount of certain pteridines (probable precursors of the red pigment) in the variegated eyes clearly demonstrate the accumulation of particular pteridines in these eyes in excess of the amount present in wild-type eyes. This fact, in conjunction with chromatographic studies on comparable mosaic eyes produced by somatic loss of an X fragment containing w^+, provides some rather critical evidence as to whether the clonal appearance of the mutant areas in many of the variegated eyes (caused by position effect) is the result of gene mutation or whether it is based on altered gene action in a given region of somatic tissue.

BANGHAM, JEAN W. *See* RUSSELL, W. L., *et al.*

BAKHTEYEV, F. KH., and E. M. DAREVSKAYA. Spontaneous and Artificial Hybridization in the Genus *Hordeum* L. *Botanical Institute of the Academy of Science, USSR, Leningrad, U.S.S.R.*

The findings obtained from spontaneous interspecific and intergeneric hybrids and the results of experimental investigations on remote hybridization show that hybridization is one of the most striking and efficient factors in plant evolution.

The genus *Hordeum*, family Gramineae, includes, according to Nevsky, only 28 species, combined into 6 sections. The section Crithe Döll includes all cultivated barleys and one of their nearest relatives, the wild-growing annual *Hordeum spontaneum* C. Koch. In the U.S.S.R. 11 wild-growing *Hordeum* species are registered. As a result of many years of investigation, we distinguish three species among cultivated barleys: *H. vulgare* (L.) Vav. et Bacht., *H. aethiopicum* Vav. et Bacht., and *H. humile* Vav. et Bacht. The occurrence of natural hybrids among wild-growing species of barley have not been recorded. Artificial hybridization was begun by G. Bestehorn as early as the 1880's and still continues. Over 20 cases of hybridization between *H. bulbosum, H. jubatum, H. nodosum, H. brevisubulatum, H. murichum* and cultivated barley species are known. As a result of artificial crosses between barley and species of *Elymus*, *Secale*, *Agropyron*, *Bromus*, *Festuca* (started under the direction of Zizin in the thirties) occasional, absolutely

sterile intergeneric hybrids were obtained, particularly a hybrid between cultivated barley and *Elymus giganteus*, *Elymohordeum Zizinii*, Bacht. et Dar.

The overwhelming majority of crosses between members of different species, belonging to different genera or even to the same genus, fail. Nevertheless, successful instances of remote hybridization prove the possibility that further studies may overcome incrossability. Overcoming sterility is probably the most difficult problem. This is confirmed by exceedingly rare cases, where F_1 hybrids were transformed into fertile ones. However, studies conducted during the last decade show that persistent experimental work in this direction, based on the use and further development of modern cytogenetical methods, may greatly contribute to the overcoming of remote hybrid sterility.

Barber, Horace Newton. The Processes of Natural Selection. *University of Tasmania, Hobart, Tasmania, Australia.*

The method of estimating the magnitude of selective forces in natural populations are the *dynamic*, where changes in gene-frequency over a known time are measured, and the *static* where conditions determining equilibrium frequencies of genes are examined. Both methods have been used to measure the Darwinian fitness operating in populations of Tasmanian organisms. They include the following.

(1) *Oryctolagus*. Since the introduction of rabbits to northwest Tasmania 40 years ago, a cline in the frequency of blacks, which rises from $<1\%$ on the coast to about 30% 20 miles inland, has developed. On the coast the dominant wild-type allele probably has the greater fitness, whilst inland its fitness falls to 80–95%. Similar clines for coat-colour genes occur in the native marsupial *Trichosurus*. In both animals the black is favoured in areas of high rainfall carrying southern rain-forest.

(2) *Eucalyptus*. (*a*) In clearings maintained for 40 years under transmission lines, changes in frequencies of genes controlling flavonoid production in young leaves have occurred in eucalypt coppice and saplings. Preliminary estimates indicate about a 5% increase in the fitness of red as compared with yellow-green when the forest canopy is removed. In untouched forest there is evidence of a cyclical change in fitness, the fitness of genes for red flavonoid gradually decreasing as the canopy matures. This cycle of selection maintains a true morphic balance over wide areas.

(*b*) A comparison of frequencies of waxy-glaucous plants in adult forest with those in seedlings produced from mothers along steep altitude clines has given direct evidence that in *E. urnigera* genes for wax-production are effectively "lethal" at the lower altitude limit (2000 ft.); 1000 ft. higher such genes are essential for survival.

(3) In *white human populations* established 100–150 years ago, Scott has shown that a clinical pattern of performance in a standard ability test has appeared. Children of settlers in marginal lands (which usually coincide with black rabbit country) have a consistently lower performanec than children in good agricultural areas or towns. It is suggested that differential migration is the explanation of the origin of the cline.

In all these cases, fitnesses change rapidly and greatly in both space and time. The evolutionary implications of such systems of selection will be discussed, in

relation to sampling "drift," the origin of correlated variation (often interpreted as "introgression") and the possibilities of sympatric speciation.

BATEMAN, ANGUS JOHN. The Two Classes of Dominant Lethal in the Mouse. *Christie Hospital and Holt Radium Institute, Manchester, England.*

When a female mouse is dissected on the thirteenth day of pregnancy, any dominant lethals will be recognisable either as a discrepancy between the numbers of corpora lutea and implantations, or as deciduomata. Later deaths are infrequent and seem to bear no relation to the mutation rate. Sections of deciduomata show no sign of embryo, even as early as the fifth day. On the other hand, all eggs, even unfertilised ones, are still present in their zonae pellucidae on the fourth day. It follows that all dominant lethal ova disappear within a period of about 24 hours around the time of implantation. During this period some invoke a deciduomatal reaction by the endometrium and others do not. The partition between these two phenotypes of dominant lethal is not a matter of pure chance, as it varies systematically with the over-all incidence of lethals.

Study of this variation leads to the conclusion that it is consistent with the hypothesis that all eggs with a single lethal, and a constant fraction (about one-quarter) of those with more than one lethal, invoke a deciduomatal response. It is hoped to distinguish two classes of lethal cytologically.

The partition between the two types of lethal follows the same pattern for X-rays and triethylenemelamine, implying that a Poisson distribution of breaks per egg results from both mutagens.

BATEMAN, NIGEL. Response of Mice to Selection for Lactation. *A.R.C. Animal Breeding Research Organisation, Roslin, Scotland.*

Selection for lactation performance in mice has been carried in both upward and downward directions through 20 generations. A number of reverse selections were made in parallel with the two forward lines to assess the magnitude of heritable variation within these lines. The responses within these single populations to the two-directional selections indicated that heritability was 0.5 and did not change during the experiment. Somewhat lower estimates of heritability were obtained from the variance between full-sib families, and from the regression of daughter on dam, computed within selection groups.

It is doubtful whether the high line responded to the selection, and the response of the low line was not maintained at its initial level. The earliest generations of the high and low lines diverged in accordance with the estimated heritability of 0.5, but by the twentieth generation the realized heritability had diminished to 0.17; i.e., only one-sixth of the total selection applied up to the twentieth generation appeared as a difference between the two lines. The inference is that the response to reverse selection averaged 0.83. This corresponds to a five-fold change in response following a change in the direction of selection.

It appears that a natural mechanism (as yet unidentified) opposes departure from a particular level of lactational performance. However, the lactations of reciprocal crossbreds between the two lines are identical, and this excludes maternal effects as explanation.

BATTAGLIA, BRUNO. Polymorphism and Relationships with the Environment in *Tisbe reticulata*. *Istituto di Zoologia dell'Università, Padova, Italy.*

The harpacticoid copepod *Tisbe reticulata* is a polymorphic species. As previously described by Bocquet, the various forms are characterized by a different distribution and intensity of the colored pigment in the cephalo-thorax and the free thoracic segments. In the brackish waters of the lagoon of Venice four major forms have been found. The results of crosses made in the laboratory show that the color patterns in the animals of this population are due to a series of at least three allelomorphs at the same locus: V^V, V^M and v, of which the first two are dominant over the common recessive v. The heterozygote V^V V^M *is* phenotypically detectable.

Under optimal culture conditions, in which larval mortality is practically absent, the observed ratios in the F_2 of crosses between V^V and V^M agree with the expected Mendelian ratios. However when death occurs, during the period egg-adult, an excess of heterozygotes V^V V^M and a deficiency of both homozygotes V^V V^V and V^M V^M can be observed. There is some indirect evidence that the heterozygotes V^V v and V^M v may also, under certain conditions, be more viable than both the respective homozygotes. The viability coefficients are modified by the degree of crowding and therefore by the larval competition in the cultures. The greater the crowding, the more the numbers of heterozygotes exceed the expected values. Salinity is another environmental factor affecting the adaptive values of the different genotypes. At a salinity of 26‰ the homozygotes V^M V^M have a selective advantage over the homozygotes V^V V^V and the heterozygotes V^V V^M. At lower salinities selection favours the heterozygotes. Other experiments now in progress will show whether temperature also plays a selective role. The results obtained so far indicate that in *Tisbe* heterosis acts to ensure the maintenance of genetic variability, in addition to other mechanisms which operate through a differential adaptation of the various forms to different environments.

BEARDMORE, J. A., TH. DOBZHANSKY, and O. PAVLOVSKY. Adaptive Polymorphism and Developmental Homeostasis in Populations of *Drosophila pseudoobscura*. *Department of Zoology, Columbia University, New York, N.Y., U.S.A.*

Seven populations of *Drosophila pseudoobscura* were started and maintained in cages at 25°C as follows: 2 containing the AR gene arrangement; 2 containing the CH gene arrangement; and 3 containing both AR and CH (initially 50:50).

After eight months (equilibrium attained), individual cups with pupae were removed from cages and the emerging flies were classified as to several characters: (*a*) body weight; (*b*) wing length (both); (*c*) number of flies emerging per cup.

The flies from cups in cages containing both gene arrangements were heavier and less variable in weight, had longer and possibly less variable wings, were less asymmetrical in the difference (intra-individual) between left and right wings, and were produced in greater numbers than those flies from cups in cages containing only one gene arrangement.

It is well known that in general the chromosomal polymorphism found in *D. pseudoobscura* persists in a variety of environments owing to the heterotic superiority of gene arrangement heterozygotes. This heterosis is most obvious in the competitive larval stages and leads to an excess of adult heterozygotes in populations. These results show that populations in which at most half the individuals are

heterozygous are on average superior in a number of ways to homozygous populations. This superiority is expressed as an increase in general biological efficiency both within and among individuals. In other words, there is good correlation between heterosis and good homeostasis although the correlation varies for different characters. We do not as yet know what developmental reactions there may be between heterozygotes and homozygotes raised in the same culture, it is however possible that the observed superiority does not have a strictly additive basis but involves inter-genotype interactions.

BEARDMORE, J. A. *See* VETUKHIY, M. O.

BEARDMORE, JOHN A. *See* LEVINE, LOUIS.

BEATTY, ALVIN V., and JEANNE W. BEATTY. Cellular Mechanisms Involved in Radiation-Induced Chromosomal Aberrations. *Emory University, Georgia, U.S.A.*

These investigations are concerned with the source of energy for repair and recovery from radiation damage. The first microspore division of *Tradescantia paludosa* is being used as the biological material, X-rays (usually a total dose of 400 r) provide the source of radiation and the experimental variables include the use of oxygen at various concentrations, anoxia, different temperatures from 0°C to 45°C, carbon monoxide, sodium iodoacetate, and p-chloromercuribenzoate. It has been found, for example, that 400 r of X-radiation, administered at any intensity to material in 5% oxygen, will yield about 0.90 aberrations per cell when the energy-supplying system is inhibited by carbon monoxide, low temperatures or both. In an oxygen-free atmosphere a maximum of 0.65 aberrations per cell has been obtained following an anoxic condition extended to 400 minutes. Immediate radiation after the material is placed in an oxygen-free atmosphere yields only 0.24 aberrations per cell. Although sodium iodoacetate (M/1,000) and p-chloromercuribenzoate (M/10,000) when used as anaerobic inhibitors increase the aberration yield over controls about a hundredfold, the yield does not equal that produced with 400 min. of anoxia. Other concentrations of these agents and other methods of treatment are being employed.

BEATTY, JEANNE W. *See* BEATTY, ALVIN V.

BEATTY, RICHARD ALAN. Genetics of Mammalian Spermatozoa. *Agricultural Research Council Unit of Animal Genetics, Institute of Animal Genetics, Edinburgh, Scotland.*

The characteristics of mammalian spermatozoa can be regarded, from one point of view, as somatic characters of the male that are potentially subject to genetic influences conditioned by the diploid genotype of the male. It has been found (with

the assistance of R. A. N. Napier) that the mean size of spermatozoon head varied widely in six genetic groups of rabbits that were more or less unrelated for the preceding four generations. Marked differences in the average size of the spermatozoa were also evident in eight inbred strains of mice (in association with N. K. Sharma).

From another view-point, the spermatozoa may be regarded as a haploid generation amongst whose individuals the genes of the immediately preceding diploid generation have been partitioned during meiosis. There might, therefore, be differences among spermatozoa attributable to their own haploid gene complements: i.e., the results of meiotic segregation might be demonstrable in the phenotype of spermatozoa (Beatty, Proc. Roy. Physical Soc. *25*: 39). Some preliminary results have been obtained in current attempts to demonstrate such phenotypic differences, with particular attention to segregations at a single locus, and to the segregation of X and Y chromosomes. Techniques for the statistical evaluation of bimodal curves have been explored.

The practical stimulus for this work is that if meiotic segregation is reflected in the phenotype of the spermatozoa then the possibility arises of artificial separation or of differential inactivation of spermatozoa according to their genotype. A more fundamental interest is that the genetics of the haplophase may have repercussions on the genetics of the diplophase.

Beaudry, Jean-R. Contribution to the Study of the Process of Evolution in the Genus *Solidago* L. (Compositae: Astereae). *Institut Botanique, Montréal, Canada.*

Gradual speciation, polyploidy and hybridization are three factors which have undoubtedly participated in the evolution of the genus *Solidago*. Chromosome number determinations have shown that the basic number of this genus is nine. Since most of the species studied are diploid, it is evident that gradual speciation through selection of mutations and recombinations has been an important factor of evolution in *Solidago*. Polyploidy has been at the origin of at least two classes of biotypes: a first one in which the entities concerned have reached a level of macroscopic morphological differentiation such that they can easily be considered as good species by the standards of competent classical taxonomists; a second one in which the biotypes are still characterized by morphological features that are entirely or almost entirely microscopic. Intermediates between these two classes of biotypes evidently exist. Taxonomists have often reported hybrids in *Solidago*, but very little is positively known about their evolutionary contributions. However, comparative studies of various taxa on the basis of their morphology and chromosome numbers and also more detailed studies by biosystematic methods strongly point to a significant contribution by hybridization to the evolutionary history of *Solidago*.

Becker, Hans Joachim. Puffing in the Salivary Gland Chromosomes of *Drosophila melanogaster*. *Department of Zoology, University of California, Berkeley, Calif., U.S.A.*

Puffs are formed in the salivary glands of this species and are most numerous beginning about 5 hours before puparium formation. It was suspected that 108

short sections of the 5 long chromosome arms were subject to puffing because of their sometimes relatively wide and light appearance. About one-third of these sections actually form puffs, i.e., they are enlarged only at certain stages of development, whereas the remainder have more or less the same structure during all developmental stages. This was determined by investigating the chromosomes of different larval and prepupal stages, beginning 70 hours after hatching, the earliest analysable stage so far. During the last few hours of larval life, when the developmental stage of a larva cannot be determined exactly by its chronological age, use was made of the regularity with which some sections are puffed simultaneously while others are never seen puffed together. If, for example, A + B and B + C are sometimes simultaneously puffed, whereas A only in younger and C only in older stages, the sequence of occurrence with advancing development must be ABC. For the interval studied a timetable could be constructed with 18 consecutive stages, each of which is characterized by a certain puffing pattern. Each puff appears at and stays for a specific time. Some of them stay approximately one hour. Furthermore puffing differences have been found between the anterior transparent and the posterior opaque parts of the gland. Also being studied are the possible effects of various mutational changes in the length of larval life on the sequence pattern of puffing. Larvae homozygous for lethal giant larvae (*lgl*) never form any of the puffs which appear in normal larvae during the last 5 hours of larval life. Investigation of the mutant factor giant (*gt*) is in progress. (This study was aided by a grant from the National Science Foundation.)

Becker, Walter A., and Lawrence R. Berg. The Sensitivity of Chickens to Differences in Diet. *State College of Washington, Western Washington Experiment Station, Puyallup, Wash., U.S.A.*

The sensitivity of chicken populations to environmental changes was studied in relation to the concept of genetic homeostasis. The trait used was body weight. The F statistic, between environments mean square/within environments mean square, was used to measure sensitivity.

In the first experiment male and female White Rocks were each divided into two groups (N = 263) at hatching. One group was fed a diet that produced rapid growth and the other group a diet that gave slow growth. Body weights were taken at one, four, and eight weeks of age. Males were more sensitive than females and the birds were more sensitive at four weeks than at one or eight weeks.

Males of highly inbred White Leghorns, slightly inbred White Rocks, a heterozygous Synthetic breed and a White Rock female × Synthetic male cross were each divided into two dietary groups (N = 36) at hatching. Weekly weights were taken from one to eight weeks of age. The highest sensitivity occurred when the birds were two weeks old. At that age White Rocks and White Leghorns were equal in sensitivity followed by the Cross-breds and the Synthetics. All genotypes except the Cross-breds decreased steadily in sensitivity after two weeks. The Cross-breds increased in sensitivity and were more sensitive than the other genotypes at seven and eight weeks of age.

Variances on the geometric scale indicated that the inbred White Leghorns were the most variable throughout the experiment. The White Rocks had the least variability at two weeks of age while the Cross-breds had the lowest variability at seven and eight weeks. The main factors that affected sensitivity were the sex and age of a population while variability did not appear to be related to this trait.

Bednekoff, A. G., W. H. Stone, M. R. Irwin, and K. P. Link. Chemical Studies on the J Substance of Cattle Serum. *Departments of Biochemistry and Genetics, University of Wisconsin, Madison, Wis., U.S.A.*

The J substance of cattle blood has been described as a heritable antigenic character present primarily in serum and acquired by erythrocytes. The serological and immunochemical properties of the J substance obtained from bovine gastric mucosa indicated that it was related serologically and chemically to the blood group A substance of man. The preparations from bovine gastric mucosa consisted primarily of polysaccharides similar to those previously described for the blood group substances of man. Because the isolation procedure (phenol-extraction) removes protein, it is possible that protein is always associated with the J substance. The J activity of serum as measured by hemolysis inhibition was removed completely by perchloric acid, sulfosalicylic acid, trichloroacetic acid and 90% phenol, suggesting that J activity of serum is associated with protein. The J activity of serum and of gastric mucosa preparations was destroyed by sodium meta-periodate and periodic acid, suggesting further that polysaccharide is involved in J activity. Electrophoresis of untreated J active serum indicated that the J substance is in the α_1 and α_2 globulin fraction of the serum proteins. However when serum was fractionated by an alcohol precipitation method (Cohn "10") all of the J activity was found in a single fraction which contained six components by electrophoresis. The J substance of serum is remarkably heat stable. After heating at 98°–100° C for 15 minutes, the serum lost about 90% of the original protein but had nearly the same J activity as the untreated serum. Electrophoresis of this heat stable fraction showed four components; the largest component (60%) had the mobility of an α globulin and possessed about 70% of the activity of the untreated serum. These data indicate that the J substance of bovine serum is a glycoprotein with electrophoretic mobility similar to the α globulins of serum.

Belgovsky, M. L. The Shape of Frequency-Dosage Curve for Recessive Lethals in *Drosophila* in Relation to Differential Radiosensitivity of Different Stages of Germ Cell Development. *Institute of Biophysics, Academy of Sciences of the USSR, Moscow, U.S.S.R.*

The shape of the frequency-dosage curve for radiation-induced recessive lethals in *Drosophila* was one of the main facts on which the conception of the mechanism of mutagenic action of ionizing radiations was founded. After the discovery of differentional radiosensitivity of different stages of gamete development in *Drosophila* it became clear that the material used for the establishment of the shape of this curve was biologically heterogenous inasmuch as it consisted of cells of different radiosensitivity. Muller with co-workers has shown that the frequency of lethals induced in spermatids increases much more slowly than does the dose of radiation. This raises the question, why does the integral frequency-dosage curve for lethals retain its linearity?

In order to answer this question, we treated wild-type *Drosophila* males with 1,000 and 4,000 r of X-rays and recorded the frequency of recessive lethals in gametes, which at the time of irradiation were at the stages of mature sperms, spermatids and spermatogonia, correspondingly. In spermatids 4.3% of induced lethals were recorded at 1,000 r and 6.64% at 4,000 r, a fourfold increase of the

dose raising the frequency of mutations only by a factor of 1.54. These data are in agreement with those of Muller *et al.* At the same time the frequencies of lethals in mature sperms were found to be 1.79% and 5.96%, correspondingly. These figures do not significantly deviate from those expected in the case of linear dependence, with due allowance for the saturation effect.

It is thus obvious that the shape of the integral frequency-dosage curve for lethals is wholly determined by the mode of dependence upon dosage of the frequency of lethals in mature sperms. The lagging of the lethal frequency in spermatids behind that expected on the basis of a direct proportionality rule practically is not at all reflected on the shape of the integral curve. This is evidently to be explained by the fact that the absolute number of lethals originating in spermatids is very small, as compared with that of lethals originating in mature sperms, because at any given moment the latter are much more numerous than the former.

This study shows that the possible heterogenity of the stage of development of the treated germ cells, not considered in many previous investigations of the dependence of mutation frequency upon the dose of radiation, does not invalidate the conclusions drawn from such investigations, provided these conclusions refer to mature sperms only.

BELIAEVA, V. N. *See* PROKOFIEVA-BELGOVSKAYA, ALEXANDRA, *et al.*

BELL, A. E., and C. H. MOORE. Further Comparisons of Reciprocal Recurrent Selection with Conventional Methods of Selection for the Improvement of Quantitative Characteristics. *Poultry Science Department, Purdue University, Lafayette, Indiana, U.S.A.*

Reciprocal recurrent selection (RRS) was compared in two experiments with individual and family selection (IFS) for improving a highly heritable trait, body weight in the flour beetle, *Tribolium castaneum.* In both experiments the two methods were equalized on the basis of population size (56 families with 20 males and 20 females weighed each generation). By limiting selection to sire testing under RRS, generation cycles were maintained simultaneous with IFS. Inbred lines were developed from each of the 8 common foundation stocks for subsequent comparisons in hybrid combinations with populations under RRS and IFS. For control lines, the initial closed populations were reproduced each generation by random mating.

The initial mean body weights in micrograms were practically equal for all populations at 2,150 for males and 2,300 for females with a standard deviation of 250 micrograms. Heritability of body weight in the initial populations was high (.60–.80) and the superiority of IFS was apparent in both experiments by the third generation. After sixteen generations of selection in both experiments, representative inbred lines were crossed in hybrid combinations to yield the following comparisons of mean body weight of both sexes in micrograms: Experiment I—IFS = 4,683, RRS = 3,002, largest hybrid = 2,936, $\bar{x}$ of 11 hybrids = 2,381, control = 2,265; Experiment II—IFS = 4,561, RRS = 2,999, largest hybrid = 2,812, $\bar{x}$ of 10 hybrids = 2,363, control = 2,187. Much of the superiority shown by the closed population method is a function of a larger selection differential which was inherent in the design. These results confirm our earlier *Drosophila* findings in showing that reciprocal recurrent selection is an inefficient method for improving

quantitative traits influenced largely by additive gene effects. Its role in improving heterotic traits has yet to be clearly delineated. (This work was supported by grants from the Rockefeller Foundation and the National Science Foundation.)

BELL, A. E. *See* MARTIN, G. A.

BELLUOMINI, H. W. *See* SCHREIBER, GIORGIO, *e tal.*

BENDER, HARVEY A., and ROBERT C. KING. Studies on the Expression of Various *Singed* Alleles in *Drosophila melanogaster. Department of Biological Sciences, Northwestern University, Evanston, Ill., U.S.A.*

The 14 *singed* stocks of *Drosophila melanogaster* studied can be subdivided into four categories: (1) fertile, with kinked hairs and gnarled bristles—sn^3; (2) fertile, with kinked bristles only—sn^2, sn^4, sn^{34e}; (3) sterile, with kinked hairs and gnarled bristles—sn^1, sn^5, sn^{50k}, sn^1sn^2, sn^1sn^3, sn^1sn^4, sn^2sn^3, sn^{x2}, sn^c; and (4) sterile, with gnarled bristles only—sn^{36a}. The stocks containing two pseudoalleles on the same chromosome were synthesized by Professor W. M. Hexter. The ovaries of mutants of class (3) show an increased frequency of degenerating chambers and stage 14 oocytes are invariably misshapen. The ovaries of females homozygous for sn^{36a} show further abnormalities. Oogenesis usually ceases at stage 12. The nuclei of nurse cells in stages subsequent to 4 show a reduced amount and an abnormal distribution of both DNA and RNA (as measured by the Feulgen and azure B bromide procedures). Adult females were fed medium supplemented with RNA (1 part per 20 parts medium) to see if the abnormal behaviour of the nurse nuclei could be modified. No difference was observed between the ovaries from sn^{36a} females fed upon normal or supplemented media. However, added RNA appears to slow down the rate of development of post-yolk stages in $sn^{36a}/+$ flies. The effect of DNA-supplemented medium is under study. Oogenesis is normal in ovaries from flies of the following genotypes: sn^{36a}/sn^3, sn^{36a}/sn^2, sn^{36a}/sn^4, and sn^{36a}/sn^{34e}. Oogenesis resembles that found in class (3) in ovaries from sn^{36a}/sn^1, sn^{36a}/sn^1sn^2, sn^{36a}/sn^1sn^3, sn^{36a}/sn^1sn^4, and sn^{36a}/sn^2sn^3. Note that females homozygous for either sn^2 *or* sn^3 alone are fertile. Hairs are not kinked in females of the following genotype: sn^{36a}/sn^1, sn^{36a}/sn^3, sn^{36a}/sn^1sn^2, sn^{36a}/sn^1sn^3, sn^{36a}/sn^1sn^4, and sn^{36a}/sn^2sn^3. The bristles of sn^{36a}/sn^2 flies are kinked rather than gnarled. A hypothesis will be described which attempts to explain the action of this complex locus upon fertility and upon bristle and hair morphology. (Work supported by the U.S. Public Health Service, N.S.F. and USAEC.)

BENDER, MICHAEL A. A Comparison of the Rates of Chromosome Breakage Induced by X-Rays in Somatic Cells of Humans *in vitro* and of the Monkey *Ateles in vitro* and *in vivo. Department of Biology, The Johns Hopkins University, Baltimore, Maryland, U.S.A.*

Diploid cells from the second passage in tissue culture of epithelial-like cells isolated from several normal human kidneys have been exposed to doses of hard

X-rays ranging from 10 r to 200 r. At various times after irradiation these cells have been fixed and stained and then scored for chromatid-type chromosome aberrations. All of the expected types of aberration were found to occur. The induced breakage rate was found to be about 0.3 break per 100 cells per roentgen. In order to compare breakage rates *in vitro* and *in vivo*, further experiments were performed using somatic cells of the monkey *Ateles*. This animal has a diploid chromosome number of 34, which, coupled with the fact that the chromosomes are large and easily identified, makes it a very suitable experimental primate. Surgical removal of one kidney made it possible to make tissue cultures for *in vitro* studies parallel to those made with human cells, and also permitted the use of the same animal for *in vivo studies*. For the latter studies bone marrow was removed from the femur at various times after irradiation. X-ray doses of 10 to 100 r were used in these studies. The preliminary results of the *in vitro* experiments on *Ateles* cells confirm the results for human cells. The preliminary results of the *in vivo* studies show that it is possible to score properly treated bone marrow cells for chromatid-type aberrations. Although the amount of material scored to date is small, no difference between the *in vitro* and the *in vivo* experiments has become apparent, either in the breakage rates or in the types of aberration found. (This work was supported by a fellowship from the United States Public Health Service and by contracts with the United States Atomic Energy Commission.)

BENDER, MICHAEL A. *See* METTLER, LAWRENCE E.

BENNETT, J. H., and H. N. ROBSON. A Possible Genetic Basis for Kuru. *University of Adelaide, Adelaide, Australia.*

Kuru is a neurological disease involving progressive degeneration of the extrapyramidal motor part of the central nervous system and associated with marked ataxia. It has been found only in the Forei people of New Guinea, a linguistic group of about 20,000 who live in comparative isolation in a remote part of the highlands in the southeastern part of the island. There is great variation in the age of onset of the symptoms of Kuru, but once manifest the course of development of the disease seems to be uniform and death almost invariably results in from six to nine months. At present, Kuru accounts for about 60% of deaths among Forei women and about 10% of deaths in males. The collection and analysis of family records presents great difficulties. Nevertheless, several hundred families have now been recorded. The analysis of these records suggests that Kuru may have a genetic basis.

BENNETT, JACK. Sib Selection in Small Populations of *Drosophila melanogaster* Meig. *Biology Department, Northern Illinois University, DeKalb, Ill., U.S.A.*

A heterogeneous stock of *Drosophila melanogaster* Meig. was produced from 26 non-resistant laboratory and wild strains. It was proliferated in population cages and sub-populations extracted for selection. Samples of each of an initial group of 46 pair mating progenies were tested in vials lined with DDT-impregnated paper

to determine resistance to kill. The untested sibs from the 20 most resistant and the 20 least resistant progenies were used to make up 50 pair matings to start each of the prospective lines (High = resistant, Low = susceptible). Similar testing and selection was carried out on subsequent generations for each line. After the first generation each line was duplicated, thus 2 High and 2 Low lines, all from the same population, were selected. Selection was carried on for 15 generations. At the end of the period of selection the median lethal doses for the High lines were approximately 125 and 625 times as great as those for their respective paired Low lines, and approximately 25 times as great as the median lethal dose for the control population cage.

The method of sib-selection ensures that the reproductive individuals of each generation were never exposed to the selective agent. The resistance developed compares favorably with the resistance of a variety of lines from our and other laboratories that were produced by direct selection.

An analysis of the chromosomal distribution of the genetic factors for resistance and susceptibility is in progress.

BERG, LAWRENCE R. *See* BECKER, WALTER A.

BERG, R. L. The General Evolutionary Principle Underlying the Origin of Biological Homeostasis. *Department of Darwinism, Leningrad University, Leningrad, U.S.S.R.*

In living beings homeostasis is expressed by the independence of their forms and functions from the accidental variation of environmental conditions. Stability is a particular manifestation of this autonomy. An investigation was undertaken to establish the diversity of the degree of stability of various characters of species, to find out the adaptive significance of this diversity, and to reveal the mechanism leading to the origin of higher degrees of stability.

Dimensions of different parts of 20 species and varieties of angiosperms have been studied. The degree of stability is expressed by the ratio V max/V min and by the variation coefficient; the degree of independence by the correlation coefficient. The absence of correlation indicates the independence of dimensions of the organ in question from the dimensions of other parts of the organism.

Comparison of the vegetative and reproductive characteristics shows the higher stability of the latter in all the species and varieties studied. High correlation coefficients have been detected for the dimensions of stems, leaves and inflorescences. The especially high degree of stability as well as the absence of correlation has been detected for the corolla and for stamens of plants pollinated by specialized insects. In self-pollinated plants as well as in plants pollinated by unspecialized insects or inanimate agents, a relatively low degree of stability of reproductive characteristics and no independence of their dimensions are observed. Stability and independence are thus inherent in dimensions of those parts of dorsoventral laterally placed flowers which participate in the process (described by Darwin, and recently by Schwanvitsch) of precise localization of pollen on a definite part of the body of the specialized insect. Specific pollinating insects act as selective agencies with respect to dimensions of certain parts of flowers. They take no part whatever in the formation of flowers during ontogeny. In higher organisms formative and

selective factors never coincide. The lack of coincidence between formative and selective factors is the general principle underlying the origin of homeostasis. In the process of evolution this principle came into action simultaneously with the origin of the alternation of generations, and acquired special importance after the origination of ontogeny. Coding inherent in the physical basis of heredity is its direct consequence.

BERG, ROY T., and JOHN P. BOWLAND. Observations on Genotypic Response of Swine to Energy and Protein Levels in the Ration. *Department of Animal Science, University of Alberta, Edmonton, Alta., Canada.*

Knowledge regarding non-linear interactions of heredity and nutrition over practicable ranges of nutritional status would be of value in relation to the formation of appropriate test rations for use in evolving improved strains of swine. Two strains of pigs, sired by Yorkshire and Tamworth boars, were fed rations differing in energy and protein levels from initial weights of 50–60 lb. to an average market weight of 195 lb. Four male and four female pigs from each strain were self-fed each of the following rations: Ration I, high energy (80% T.D.N.)-high protein (21 per cent crude protein) throughout; Ration II, high energy-high protein in the growing period to 110 lb. and high energy-low protein (13% crude protein) in the finishing period to market weight; Ration III, low energy (65% T.D.N.)-high protein throughout; Ration IV, low energy-high protein in the growing period and low energy-low protein in the finishing period. All rations were balanced with respect to other known nutrient requirements.

In the finishing period average daily gains were significantly different between rations and between sexes at $P. < .05$ with strain $\times$ sex interaction significant at $P < .01$. Efficiency of feed utilization in the same period differed between rations, strains and sexes at $P < .01$ with ration $\times$ strain interaction significant at $P < .05$.

Carcass measurements and scores were obtained as outlined in the National Bacon Hog Policy of the Canada Department of Agriculture. Where applicable adjustment for differences in warm carcass weight was made by covariance analysis. Ration groups were significantly different in percentage ham and belly scores at $P < .05$ and in total carcass scores at $P < .01$. Sexes differed in average back fat, percentage of ham, loin area, belly score and total carcass score at $P < .01$. All first order interactions for carcass characteristics among rations, strains and sexes were negligible. The magnitude of the difference in efficiency of feed utilization between two strains of swine varied with the ration fed, while differences in carcass merit between the strains remained quite constant over the rather wide ranges of protein and energy in the rations used.

BERGH, B. O. *See* JENKINS, J. A.

BERGQUIST, ARLOA. *See* WAGNER, R. P.

BERHMANN, S. J. *See* BUETTNER-JANUSCH, JOHN, *et al.*

BERNSTEIN, SELDON E., and LEROY C. STEVENS. Jaundice, a Congenital Microcytic Anemia of the House Mouse. *Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.*

A number of interesting hematological mutants are currently under study at the Jackson Memorial Laboratory. Jaundice, one of these, was recently discovered at this laboratory by Dr. Stevens. His studies indicate that the disease is inherited as a single Mendelian recessive, and that the homozygous recessive genotype dies shortly after birth. Ratios of normal to anemic embryos and newborns do not deviate significantly from the classical ratios although the diagnosis is complicated by the fact that some mice are born dead.

This condition which is invariably fatal within a few days of birth is characterized by a yellow coloration of the skin which becomes grossly visible within 6 hours of birth. *In utero* the affected offspring are pale but not yellow. Their livers, however, are large and congested, and soon after birth splenomegaly and cardiac enlargement become prominent features.

Analyses of the blood indicate a severe anemia, newborn *jaja* mice have average erythrocyte counts of 2×10^6 RBC/ mm.^{3} while counts of their normal sibs average 4×10^6 RBC/ mm.^{3}. The mean cell volumes of *jaja*'s range from 40–70 cubic microns as compared with 100–120 cubic microns for non-affected sibs. Large numbers of nucleated red cells, up to 400,000/mm.^{3} are found in the circulating blood, while many of the enucleated forms are bizarre in shape and staining capacity.

Morphologically the heterozygotes are indistinguishable from homozygous normals at birth and in adult life. The detection of these carriers so that production of jaundiced mice may be facilitated, and increasing the viability of the latter are two of the main problems under investigation. The report deals largely with these attempts, gross observations, and a comparison of jaundice with human and other murine anemias.

BERNSTEIN, SELDON E. *See* RUSSELL, ELIZABETH S., *et al.*

BHAT, N. R. *See* MURTY, B. RADHAKRISHNA.

BIANCHI, ANGELO. Genetic and Cytological Studies in Italian Maize Varieties. *Centro di Genetica, Università di Pavia, Pavia, Italy.*

To detect mutants in Italian maize varieties, self-pollination has been carried out in plants grown from seed collected throughout Italy. With the exception of plant colour characters the segregations were often very close to 3:1; in a few cases the ratio was 15:1. In a total of 290 selfed plants belonging to 170 different samples of open-pollinated populations, the following mutants have been observed: defective seed (12 types), sugary endosperm (1); virescent (24); glossy (20); albino (18); luteus (12); yellow-green (11); yellow stripe (6); abnormal growth (6); pale green (5); liguleless (4); fine stripe (3); brownish midrib (2); irregular (necrotic?) glossy (2); pigmy (1). Out of the 12 defective seed factors observed just

one is from Northern Italy, which however contributed about two-thirds of the samples studied. Several weak alleles of the tunicate type have been collected, especially from Middle and Southern Italy. Cytological study dealt chiefly with the knob number and knob location. The average knob figure is low and never exceeds four. The identification of the single knobs has been possible in most cases. The most frequently observed knobs were on the long arms of chromosomes 7 and 8, and on the termination of the short arm of 9. No abnormal chromosome 10 has been observed; the heteropicnotic region close to the centromere in the long arm of 7 is often difficult to detect; a prominent chromomere may be observed following the knob in the long arm of 8, closer to it than in American strains. B chromosomes have been found in one plant only. Incomplete synapsis or precocious desynapsis was occasionally present in the pachytene chromosomes. In two plants, metaphase I showed few bivalents and several univalents. The chromosome pachytene spreading as well as the staining was quite variable. Very clumped type of pachytene configuration, however, has not yet been found.

BIANCO, I. *See* SINISCALCO, MARCELLO, *et al.*

BISHOP, C. J. Radiation-Induced Morphological Changes and Fruit Color Mutations in the Cortland Apple. *Canada Department of Agriculture, Experimental Farm, Kentville, Nova Scotia, Canada.*

Following a brief survey of the history of using radiation to induce morphological and genetic changes in agriculture, work at Kentville, using X-rays and thermal neutrons on apples, is discussed and summarized. About 13,000 dormant scions have been treated and then grafted into grown trees by the framework method. Dosages ranged from 3,500 to 5,000 roentgens of X-rays and 4 to 6 $\times$ 10^{12} thermal neutrons per cm.^{2}.

Morphological effects in growth from scions of the variety Cortland included distorted leaves, splits in leaf shape and veining, bifurcations of growing shoots and giant size of the first fruits. The latter may have been a physiological effect due to the grafting procedure. Genetic changes included frequent sectorial chimaeras in skin color, increased or decreased red color, and at least two branches on which the apples were complete sports and of a dark red color. The latter were very distinctive in the shade of red, being as dark in color as the variety Macoun.

The results obtained indicate that artificial radiation may become an important tool in apple breeding research. It is necessary, however, to process large numbers of scions to have a reasonable chance of success.

BLICKENSTAFF, JOYCE, PATRICIA SARVELLA, and R. A. NILAN. Pachytene Chromosome Analysis in Barley. *State College of Washington, Pullman, Wash., U.S.A.*

The genetics of barley has been studied more intensively and the linkage groups characterized by more known loci than most crop plants. The extreme difficulty of identifying individual chromosomes at mitosis and meiosis has hindered the assigning of linkage groups to specific chromosomes. Study of pachytene chromosomes

in varieties and strains previously examined has been particularly unrewarding because of unsatisfactory spreading of the complement and staining of the chromomeres.

Microsporocytes of fertile lines derived from certain heterozygotes of the short chromosome (*sc*) strain of barley ($n = 7$) have proved to be favorable for pachytene analyses. Short chromosome is a meiotic mutant (Moh and Nilan, Cytologia *19*:48–53) which is characterized by extremely condensed diakinesis chromosomes and pollen sterility. In addition, the mutant exhibits relatively well-spread pachytene chromosomes. The selected fertile lines appear normal at diakinesis but retain the well-spread pachytene condition.

The problems of chromosome spreading and staining have been alleviated by examining freshly fixed (4 parts alcohol : 1 part acetic acid) microsporocytes that were subsequently stained in acetocarmine and smeared following steaming.

Except for the two satellited chromosomes, there appear to be no major distinguishing characteristics in the pachytene complement. Relative lengths and arm ratios are being used as the chief means of identifying the non-satellited chromosomes. The pachytene measurements have been compared with those obtained from somatic chromosomes of the same plants. Chromomere size, number, and pattern as well as tertiary constrictions are also being used to distinguish the different pachytene chromosomes.

In order to associate each linkage group with a specific chromosome, translocation tester stocks have been crossed to the selected lines.

Blumberg, Baruch S. Studies on the Biochemical Genetics of Cattle: The Whey Proteins and Hemoglobins. *National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.*

Aschaffenburg and Drewry have reported that there are two kinds of β-lactoglobulins in the whey proteins of cow's milk. These can be separated by paper electrophoresis and their presence is determined by allelic autosomal genes. All three of the phenotypes, designated β_A, β_B, and β_{AB}, have been identified. We have studied the distribution of these proteins in herds of British, American, Icelandic and African (Zebu) cattle. During the course of these studies, it was noted that there were two types of α-lactalbumins in the milk of the African Zebu cattle which could be differentiated by paper electrophoresis. There is some evidence that these might be genetically determined. Among the breeds studied to date, the Zebu appear to be the only ones which have these two α-lactalbumins. Preliminary studies on the starch gel electrophoresis of whey proteins will be reported.

Bangham has reported on the existence of two types of hemoglobins in certain breeds of British and European cattle. Among British breeds, only the Jerseys, Guernseys and South Devons have this polymorphism. We studied the distribution of this trait in African herds. Of the five breeds of cattle studied, Nigerian, Zebu ("White Fulani"), Sudanese Zebu, Keteku (which are crosses with the Zebu), Muturu, and N'Dama, only the Zebu and Keteku had both kinds of hemoglobin. The last two breeds are relatively tolerant to trypanosomiasis, while Zebus are not. Whether this *prima facie* correlation with trypanosomiasis tolerance is more than coincidence will have to be determined by further investigation.

The significance of these biochemical polymorphisms will be discussed. Some

comments will also be made on the relationship of these findings to the supposed affinity between Jersey cattle and the cattle of India.

(These studies were done, in part, in conjunction with Drs. M. P. Tombs and A. C. Bangham.)

BLUMEL, JOHANNA, E. BURKE EVANS, and G. W. N. EGGERS. Absence of the Sacrum and Coccyx: A Genetic and Clinical Study. *Orthopedic Division, Department of Surgery, University of Texas Medical Branch, Galveston, Texas, U.S.A.*

Six cases of sacral and coccygeal anomalies in our clinic prompted us to initiate this investigation. A review of the literature indicated that the number of published cases by no means reflected the actual occurrence rate of these malformations. In addition, it was apparent that no genetic studies had been attempted. A complete review of the literature and compilation of statistics on congenital defects of sacrum and coccyx was therefore undertaken, and in March 1957 survey forms were sent to 300 orthopedic surgeons in the United States and Honolulu. As of March 1958 replies have been received from 63, 20 of whom had treated from 1 to 6 such cases. So far a total of 36 cases have been obtained in this manner. It was subsequently decided to survey each state by sending forms to hospital record librarians. In Texas 100 hospitals have been contacted. To date 32 have replied and 4 of these have furnished a total of 8 cases. The other states are now being surveyed. Results on 50 cases, including our own, with special reference to manifestations of hereditary abnormalities in collaterals and ascendants, are as follows: Sex distribution is 23 males, and 27 females. These include 2 negroes, 4 Latin Americans, 38 whites and 6 not stated. Of these 30 showed complete absence of sacrum and coccyx; 16 had partial absence and on 4 information was incomplete. Common associated congenital anomalies occurred in 34 index cases. These were: (1) additional vertebral anomalies, 24; (2) spina bifida occulta, 9; (3) arthrogryposis, 6; (4) harelip and or cleft palate, 3; (5) dislocated hips, 13; (6) club feet, 23; (7) congenital heart disease, 2; (8) strabismus, 2; (9) bladder and bowel involvement, 14. Deliveries of propositi were breech in 8 cases and 5 listed abortions numbering from 1 to 3 per family. In 8 case histories, congenital anomalies were present among siblings, maternal and paternal relations.

BÖHME, HELMUT, and UDO TAUBENECK. The Action of Bacteriophages on Rod-Cells and Penicillin-Induced Large Bodies of *Proteus mirabilis*. *Institut für Kulturpflanzenforschung* and *Institut für Mikrobiologie und experimentelle Therapie der Deutschen Akademie der Wissenschaften zu Berlin, Gatersleben, Kreis Aschersleben, Germany.*

In liquid culture medium with high concentrations of penicillin *Proteus mirabilis* develops spherical large bodies which are able to return to the normal rod-like form after artificial removal of the antibiotic. In connection with studies on phage-resistance of *P. mirabilis* the reaction of normal rod-cells and large bodies to the action of bacteriophages has been compared. Lysis of rod-cells grown in an antibiotic-free medium begins 21 minutes after initiation of adsorption of phages with a multiplicity of 5; it is completed after 30–40 minutes. The behaviour of a culture of penicillin-induced large bodies (6 to 8 hours old) after adsorption of phages with

the same multiplicity is exactly like that of the culture of rod-cells. The latent period lasts 21 minutes too. Microscopic control showed that the large bodies were lysed completely. It is easy to isolate phage-resistant mutants of *P. mirabilis* by cultivation of the sensitive strain on solid media together with an excess of phages. Penicillin-induced large bodies of a phage-resistant mutant preserve phage-resistance; no lysis occurs. The experiments were carried out with 2 unrelated strains of *P. mirabilis* and their phage-resistant mutants. The results were identical for both strains. The sensitivity to phages of large bodies induced from phage-sensitive rod-cells leads to the conclusion that the receptors necessary for adsorption of phages remain intact after transformation of rod-cells into large bodies. This is a difference between large bodies of *Proteus* and protoplasts produced from *E. coli* and *B. megaterium* which generally are not able to adsorb phages (for exceptions see Lederberg and Claire, Frazer and Mahler). The resistance to streptomycin of large bodies of *Proteus* and protoplasts of *E. coli* also seems to be different. According to Lederberg and Claire penicillin-induced protoplasts of a streptomycin-resistant mutant of *E. coli* were inhibited by a streptomycin concentration of 20 μg./ml. In our experiments the penicillin-induced large bodies of *P. mirabilis* behaved in liquid media with 400 iu penicillin per ml. plus 40 u streptomycin per ml. in the same manner as in this medium without streptomycin; that means they were not inhibited. On solid media with serum, penicillin and streptomycin the streptomycin-resistant mutant forms L-colonies as on the same medium without streptomycin.

BONNER, DAVID M. *See* LACY, ANN MATHEWS.

BOOTHROYD, E. R. The Chromosomes of Canadian Populations of Atlantic Salmon, *Salmo salar* L. *Department of Genetics, McGill University, Montreal, Canada.*

Chromosome studies were undertaken in the hope that they might throw some light on the relationships between various Canadian populations of salmon, as well as between these and European salmon.

Three populations were studied: a spring-run population from Maria, in Gaspé, Que., and two fall-run populations, one from the Miramichi River, N.B., and the other from Cobequid, N.S. Acetocarmine squashes of young embryos were used.

Counts were somewhat variable because of the difficulty of the material, but all three Canadian populations showed modes of 56 chromosomes. It is concluded, therefore, that $2n = 56$ is the true number in these salmon. Both Prokofieva and Svärdson have reported $2n = 60$ for European populations.

The majority of chromosomes have terminal or sub-terminal centromeres. The longest of these rod-chromosomes is about four times the length of the shortest. The assignment of these chromosomes to their homologous pairs is usually not possible because the length differences are too small and the appearance of secondary constrictions (up to four in some chromosomes) is too variable to be reliable. Seven or probably eight pairs of chromosomes have median or sub-median centromeres, and of these two-armed chromosomes the longest is approximately twice as long as the shortest. No clear-cut differences in either the number or the morphology of the chromosomes have been established between the Canadian populations.

These Canadian salmon do not fit Svärdson's theory that the Salmonidae represent an old polyploid series, $x = 10$, in which no centric fusion or misdivision has occurred. However, if the Canadian salmon have eight pairs of two-armed chromosomes and $2n = 56$ and if the European have six pairs and $2n = 60$, as reported by both Prokofieva and Svärdson, then one may have evolved from the other by two centric fusions or misdivisions.

Borojević, Slavko. The Occurrence on Wheat of Branched Ears in the Crosses of Species with Unbranched Ears. *Faculty of Agriculture, Novi Sad, Yugoslavia.*

In the crosses of two common wheat varieties with *Tr. dicoccum*, none of them having branched ear character, some segregates showed branched ears in the F_4 and F_5 generations. In succeeding generations the branched ears segregate in branched (majority) and unbranched ears. The ratio of segregation was quite irregular. The branching was of a turgidum- and Vavilovii-like type.

All plants with branched ears belong to 14-chromosome species, turgidum or durum types. The majority of them have regular meiosis and only a few had a smaller number of univalents which disappeared in later generations. The sterility of branched ears, however, was high, particularly of the side ears. The plants with branched ears had a longer growing season than either parent. It was also observed that the side ears developed later than the central ear. Often the central ear was out of the shoot before the side ears appeared. The intensity of branching varied from year to year, in some years being quite pronounced, while in other years the side ears had only a few spikelets.

The occurrence of the branched ears in the crosses of the parents with unbranched ears has been explained as an interaction between the species used, expressed and influenced mainly by the environment. The day length and temperatures varied greatly in the years when the progeny of these crosses and especially the progeny of the branched ears from the F_4 generation onwards were grown. Short day length and the lower temperatures are considered as the most responsible factors for the expression and stability of the branched ear character.

Bose, Smritimoy. Cytological Studies in Parental Types, and in Selfed and Crossed Progenies of *Sprekelia formosissima* Herbert. *Blandy Experimental Farm, University of Virginia, Boyce, Va., U.S.A.*

Cytological studies in *Sprekelia formosissima* Herbert have been confined to the study of chromosome number, reported by several workers to be between 110 and 121. The present investigation was undertaken to make a cytological survey of the genus from the large number of bulbs secured from several locations and from their selfed and crossed progenies.

The lowest number of $2n = 60$ was observed from bulbs collected near Cuernavaca, Mexico. Bulbs received from other sources not only had higher chromosome numbers but also exhibited a variation in numbers, in the root-tip cells. Peak frequencies were between 118–124, 148–155, and 164–178, suggesting that 120, 150 and 170 may be the regular somatic counts. Four different types of chromosomes could be distinguished in taxa with $2n = 60$ and in those with higher numbers.

F_1 seedlings of selfed and crossed plants from higher chromosome number types exhibited the same phenomenon of variation in numbers. The types of chromosomes were similar to the parental types. F_1 seedlings of $2n = 60$ taxa revealed a constant $2n$ number of 60 with parental types of chromosomes.

Repeated attempts to study meiosis, which occurs when the flower buds are still within the bulbs, failed, as did efforts to study mitosis in pollen tubes. Nevertheless a high percentage of fertile pollen and good pollen germination indicated a probable constancy of chromosome number in the pollen mother cells.

A study of chromosome types in taxa with 60 and with about 120 chromosomes apparently shows a regular increase in numbers of the several types, and indicates a polyploid series. Differences in size of the pollen grains between the two taxa could also be taken as an evidence for this hypothesis. Accessory chromosomes may possibly play a role in the cytological picture.

BOWDEN, WRAY, M. Phylogeny of Twenty-One Species of *Lobelia* L. section *Lobelia*. *Botany and Plant Pathology, Canada Agriculture, Science Service, Ottawa, Canada.*

The author's conclusions on the relationships of twenty-one species of *Lobelia* are based on correlated data derived from taxonomic, ecological, cytological, and genetic studies. A phylogenetic diagram is demonstrated and some of the voucher specimens of the species and artificial interspecific hybrids are exhibited. There are fifteen diploid ($2n = 14$) species, one species with both diploids and tetraploids (*L. nuttallii*), three tetraploid ($2n = 28$) species (*L. glandulosa*, *L. amoena*, *L. elongata*), and two hexaploid ($2n = 42$) species (*L. floridana*, *L. paludosa*). There are four lines of speciation. In each of three lines, only a single diploid species has survived: *L. dortmanna*, *L. kalmii*, and *L. inflata*. The remaining eighteen species have evoled in one complex system of speciation in which there are three distinct subgroups: (i) seven small-flowered species: *L. feayana*, *L. nuttallii*, *L. canbyi*, *L. boykinii*, *L. gattingeri*, *L. appendiculata*, and *L. spicata*; (ii) four narrow-leaved species: *L. flaccidifolia*, *L. glandulosa*, *L. floridana*, and *L. paludosa*; and (iii) seven medium- to large-flowered species: *L. georgiana*, *L. brevifolia*, *L. puberula*, *L. siphilitica*, *L. cardinalis*, *L. elongata*, and *L. amoena*.

BOWLAND, JOHN P. *See* BERG, ROY T.

BRAGDØ, MARIE. *See* HUNTER, A. W. S.

BRAGE, DIEGO. Hepatolenticular Syndrome and Familial Hyperaminoaciduria: The Expression of Homozygotes and Heterozygotes. *Docente libre de Neurologia, Facultad Medicina, Buenos Aires, Argentina.*

The author presents a study of a family with an hereditary biochemical disease. The sub-clinical cases had aminoaciduria, the manifest clinical cases had hepatolenticular degeneration with all its neurohepatometabolic symptoms.

Our proband is a 27-year-old male with the hepatolenticular syndrome. A

maternal aunt died of hepatolenticular degeneration at 30 years and a sister of the proband's maternal grandmother died of the same disease at 48 years of age.

The mother of the proband has hyperaminoaciduria. This biochemical difference was also noted in a 26-year-old sister, a 12-year-old brother of the proband, and in the son of the sole surviving descendant (a non-affected daughter) of the maternal aunt who died of hepatolenticular degeneration. The copper and protein fractions of the urine of the clinically normal people were investigated carefully and no abnormalities were detected.

This family is ethnically considered typically "criolla." All its known generations have been born and brought up in the Province of Entre Rios in the Argentina Mesopotamia. The "criollos" are the typical inhabitants of this area and were originally the products of a mixture of the Spanish conquerors and the Guarani Indians in about the 15th and 16th centuries.

The pattern of inheritance indicates that the hepatolenticular cases may be classified as homozygotes and the hyperaminoacidura cases as heterozygotes This then is a biochemical abnormality inherited perhaps as an autosomal dominant with variable expressivity. The heterozygotes may be the subclinical carriers of chemical symptoms.

It is interesting to note that copruria did not occur together with aminoaciduria which means that the latter is not secondary to the copruria.

BRAVER, GERALD. *See* CARSON, GWENETH L.

BRESLAVETS, LIDIA P. Significance of Plastids for Inheritance in Connection with the Problem of Their Origin. *Institute of Biophysics, Academy of Sciences of the USSR, Moscow, U.S.S.R.*

Some deviations from the Mendelian scheme of inheritance lead to the hypothesis of inheritance through the plasma. In the majority of cases, however, such deviations apply to the leaf coloration in the angiosperms, i.e., to the plastids. No attempts were made to connect plastid inheritance with the mode of their origin, as up to the present the hypothesis persists that plastids reproduce by division. This hypothesis is supported by the fact that the division of plastids actually takes place at all the stages of the phylogeny of plants with the exception of the angiosperms. Thorough study of this problem and the application of modern methods of cytological investigation showed that the plastids of most angiosperms have lost their ability to divide.

This is proved by the following facts. (1) The experiments in which the development of each single plastid in different cells of a leaf was followed up in an intact living plant showed the lack of division of plastids. (2) The slowness of the division of plastids in some angiosperms which preserved this ability is not in line with the rapid growth of the leaves in the angiosperms. (3) The development of plastids may proceed by another way, namely, through the transformation of mitochondria. The latter rise from the microsomes by means of the decomposition of complex organic molecules to smaller and simpler ones. (4) Inheritance of plastid characters proceeds in the angiosperms through the nucleus, with the domination of some characters in F_1 and their segregation in F_2 which was shown by cytogenetic experiments on the plastids of the petals of *Tropacolum majus* L.

The success of the angiosperms in the struggle for existence was due not only to the fact of their seeds being enclosed, but to the whole complex of characters, anatomical as well as physiological, which drastically distinguishes the angiosperms from other plant groups. To this complex there also belongs a more rapid growth of the meristem which would be hindered by the slow process of the division of the plastids.

BREWBAKER, J. L., N. SHAPIRO, and S. MAJUMDER. The Incompatibility Inhibition in Flowering Plants. *Brookhaven National Laboratory, Upton, N.Y., U.S.A.*

A great deal of attention has been focused on the genetics of incompatibility (S) alleles in flowering plants as well as on the physiology of pollen germination and growth in culture media. An attempt to wed these two approaches in order to determine the physiological nature of S-allele induced inhibitors will be described.

The inhibition leading to incompatibility takes place in at least two distinct ways: (1) the inhibition of pollen germination, peculiar to species with trinucleate pollen, and (2) inhibition of pollen-tube growth, common to species with binucleate pollen. A possible third group comprises species with binucleate pollen and hollow styles in which gametic fusion is inhibited.

In vitro studies of these types are in progress, largely employing S allele homozygotes. In each case the attempt is made to isolate allele-specific inhibitors and to minimize the effect of non-specific inhibitors (among these, sub- or super-optimal amounts of sugar and borate, sub-optimal pH, excessive concentrations of salts).

The class I inhibition of germination (e.g., broccoli, *Crepis*) has not been duplicated *in vitro*. In general such trinucleate pollens appear to have exceedingly high sugar and oxygen requirements for germination. Incompatibility is related to osmotic conditions, and often may be overcome (i.e., germination induced) by macerating stigmas or otherwise altering the stigmatic surface milieu.

There is mounting evidence that the class 2 inhibition of pollen-tube growth can be demonstrated with each of the seven alleles currently under test in petunia. Significant growth differences have been observed between pollen of incompatible and compatible genotypes when grown in extracts of styles in sugar-borate solutions. Methods of extraction, dialysis and heat lability studies, and preliminary chromatographic studies will be discussed.

It has been possible to disrupt synthesis of S-allele specific inhibitors in the style by X-irradiation. This is believed to be a possible cause of so-called "revertible mutations" which have been described for S alleles. (Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.)

BRIGGS, J. B. *See* KNIGHT, R. L.

BRILES, W. E. The Role of Cross-Reactions in the Identification of *B* Blood Group Alleles in the Chicken. *DeKalb Agricultural Association, Inc. DeKalb, Ill., U.S.A.*

The antigens of the B blood group system exhibit a wide variety of cross-reactive patterns. When exposed to a sufficiently wide spectrum of blood typing reagents,

each antigen takes on a specific pattern of agglutination. Genetic tests show that each antigen, with its complex cross-reactive pattern, is inherited as a singly segregating unit, presumably a single gene. Current data indicate that collectively the *B* blood group genes form an extensive series of alleles—over twenty-one having been reported in a recent study of twelve inbred lines by Briles, Allen, and Millen (Genetics, 1957). The analysis of the reactive patterns displayed by many different populations has revealed that few, if any, common alleles are encountered in comparing two genetically isolated breeding groups. The extreme serological and genetic diversity encountered in the B blood group system makes impractical the inclusion of such specificity differences in the antigen and allele terminology designations. A simplified system of blood group nomenclature compatible with these findings has been developed. Under this system the antigens in a population or species are designated individually by consecutive subscript numbers as B_1, B_2, B_3, B_4, etc.; the corresponding causative genes are indicated as B^1, B^2, B^3, B^4, etc.; and the reagents, regardless of their particular cross-reactive specificities, are simply designated B1, B2, B3, B4, etc., according to the antigen against which they were produced by isoimmunization. The multiplicity of cross-reactive fractions of antibodies, even in reagents prepared against the same antigen, is such that more detailed designations of reagents become quite impractical when employed widely in the species. This system of nomenclature has proved quite satisfactory in designating the blood group genotypes present in numerous commercial breeding populations and allows for expansion in time of a blood group system without necessitating revision of any of the designations for individual antigens, alleles, or reagents.

Brink, R. Alexander. The Genetic Basis of a Transallelic Change Occurring Invariably in Certain Maize Heterozygotes. *Department of Genetics, University of Wisconsin, Madison, Wis., U.S.A.*

The R^r gene in maize, which is ordinarily a stable factor conditioning self-colored aleurone, is changed invariably to a weakly pigmented form ($R^{r:st}$) in heterozygotes with stippled (R^rR^{st}). Stippled is an aleurone spotting factor, and shows no evidence of alteration in the process whereby standard R^r is changed to $R^{r:st}$ in R^rR^{st} heterozygotes. Germinally transmissible mutations of stippled to self-colored aleurone occur in this stock at the rate of about 2×10^{-3}. Ten such independently occurring self-colored mutations were tested in heterozygotes with standard R^r for ability to change standard R^r. Nine of the mutants invariably induced the change from standard R^r to $R^{r:st}$; the remaining self-colored mutant exerted no effect on standard R^r in heterozygotes. These findings exclude the R^{st} allele, as such, and self-colored mutations from stippled, as such, as the promoters of the transallelic change in standard R^r. The active element may be (1) a unique component of the R^{st} allele, or (2) the "stippling component" itself, assuming R^{st} is a compound allele comprising an anthocyanin factor and a second element, the stippling component, which determines spotting. On the second hypothesis it is necessary to assume also that (*a*) the two elements are separable by transposition, or otherwise, and (*b*) the action of the stippling component varies according to chromosome position. It could be supposed, for example, that the stippling component conjoined with the basic color factor leads to aleurone spotting, whereas even slight displacement of the former results in self-color. Similarly, the transallelic effect of the stippling component on standard R^r may occur only when the stippling

component is present at, or very close to, the *R* locus in the homologous chromosome.

BRITTON, DONALD M. Chromosome Studies in *Rubus*. *Department of Horticulture, University of Maryland, College Park, Md., U.S.A.*

Although a large number of the species of *Rubus* throughout the world have been studied cytologically, the classification of these species and the many forms remains an enigma. Chromosome number in itself is of little use as a tool because of the frequent occurrence of individuals derived from reduced fertilized eggs, as well as from fertilized unreduced eggs. Chromosome morphology has proved not to be useful because of the small size of the chromosomes. In fact this small size has led to the publication of many inaccurate determinations of chromosome number.

There are few barriers to hybridization in the genus. Even wide crosses between the diploid raspberries and the tetraploid to 12-ploid blackberries are well known.

From controlled crosses (and presumably in the wild) there are produced many dwarf plants as well as unfruitful ones. The causes of the unfruitfulness are not yet understood. Aneuploidy is considered not to be a factor in the origin of dwarfs.

Mitotic instability is another cause of phenotypic variability in the genus. Phenotypic variablity is accentuated by the fact that *Rubus* canes are *biennial*. Accordingly, each year there is an opportunity for shoots to arise from genetically unstable tissues which were derived from split divisions in these mitotically unstable plants.

BROOKS, JAMES S. An Experimental Re-examination of Mass Selection in Corn, *Zea mays* L. *Department of Agronomy, Oklahoma State University, Stillwater, Okla., U.S.A.*

In 1950 a selection experiment was initiated at Oklahoma Agricultural Experiment Station designed to supply information regarding selection procedure and to improve materials to be used in the corn breeding program.

Simultaneous selection was practised to reduce plant and ear height, to maintain the average number of nodes above and below the ear, to increase individual plant yield, to increase ear length, and to increase the average rows of grain per ear to 16 to 18 rows. Such selection was carried out on each of four open-pollinated varieties of corn during five years, using approximately 400 plants of each variety each year and selecting the ears from 20 plants for producing the next generation. Each variety was allowed to open pollinate in isolation. Records were kept on all measurements on each plant. Similar measurements were made on plants of the F_1 of a cross between two inbred lines and used as a check on environmental and seasonal variation. The selection differentials and selection progress have been determined for each year for the different characters.

Selection for reduced plant and ear height has resulted in greater height reduction than would be expected from the separate selection differentials. Node numbers have remained essentially constant during the reduction in height. The response to selection for 16–18 rows of grain per ear varied with the variety. Selection for increased ear length produced little apparent response. Selection for increased grain yield appeared to result in a decline in yield. This decline in yield in spite

of a strong positive selection differential is interpreted as the result of inbreeding depression. Evidence that inbreeding has occurred during this form of mass selection was obtained by shifting to a pedigree form of progeny selection.

BROOKS, MARGARET H. A Comparative Study of the Patterns of Development in Certain Facultative Apomicts. *Department of Agronomy, Oklahoma State University, Stillwater, Okla., U.S.A.*

Investigations of some of the manifestations of apomixis were carried out in a study of the constitution and behaviour of the embryo sacs and pollen of two closely related genera, *Dichanthium* and *Bothriochloa*, belonging to the subtribe *Amphilastrae* of the *Andropogoneae*. Use of the Bradley (1948) smear technique facilitated a survey of the frequencies of the types of embryo sacs which occur in these materials. Three ploidy levels were represented, diploid ($2n = 20$), tetraploid ($2n = 40$), and hexaploid ($2n = 60$). The diploid *Dichanthium* which reproduces by sexual means contained only one sac per ovule, all sacs being of a single type (2 synergids, 1 egg, 2 polars and a group of antipodals). The accessions of higher ploidy level are to different degrees facultative apomicts, exhibiting multiple sacs per ovule, these sacs being of two principal types. One type of sac is similar to that found in the diploid, the other being a 4-nucleate type lacking one polar and the antipodals. Comparative studies indicated that the average number of sacs per ovule and the frequency of the 2 polar type sacs is as follows: *Dichanthium*: diploid contains 1.0 sac per ovule with 100% of 2-polar type; tetraploid, 1.7 sac per ovule with 28% of 2-polar type; hexaploid, 1.2 sac per ovule with 15% of 2-polar type. *Bothriochloa*: tetraploid contains 1.7 sac per ovule with 12% of 2-polar type; hexaploid, 2.0 sac per ovule with 9.7% of 2-polar type. Similarly, a comparative study was made of the synchronization of the female and male gametes, the data indicating a more perfect timing mechanism to be operative in the *Dichanthiums*, particularly in the diploid. The precocity of initiation of embryo development in the apomicts was compared with precocity of initiation of development in the endosperm in the sexually reproducing accession. The presence of multinucleoli in various nuclei, the sizes of embryo sacs, and the occurrence of polyspermy were found to be correlated with degrees of polyploidy. It appears from these data as well as from the breeding behaviour that the *Bothriochloas* tend more strongly towards obligate apomixis in several aspects than do the *Dichanthiums*, apomixis in the former presumably being of more ancient origin. However, the finding of potential sexuality in these materials which reproduce primarily or almost entirely by apomictic means presents challenges to the utilization of both environmental controls and culture methods to increase and exploit the inherent potential.

BROWN, META S. The Implications of Pachytene and Metaphase Pairing for Species Differentiation. *Texas Agricultural Experiment Station, College Station, Tex., U.S.A.*

The genus *Gossypium* includes eighteen diploid species which fall into five genome groups on the basis of metaphase chromosome association and geographical distribution. Within a genome group, species differentiation is primarily genic, and metaphase association is reduced little if any from that within a species. In hybrids between species of different genome groups, metaphase association is

greatly reduced, and sterility is complete. Nevertheless, pachytene pairing is as close in sterile hybrids with reduced metaphase association as in fertile hybrids with good metaphase pairing, and as in species. Either pachytene pairing in hybrids is no measure of structural differentiation of chromosomes, or factors other than structural differences are responsible for reduction in chiasma formation and metaphase association.

Chromosomes paired at pachytene are equal in length despite species differences in chromosome size at metaphase. Size differences are maintained autonomously in species hybrids and in aneuploids of hybrid origin. Chiasma formation likewise may be determined in each species by a mechanism analogous to that which controls chromosome size. This mechanism alone, in the absence of structural differences, may differentiate species by reducing metaphase association. Where structural differences can be demonstrated, they are not necessarily the primary factor in species differentiation.

BRYAN, JOHN H. D., and JOHN W. GOWAN. The Regenerative Response of the Mouse Testis to Two Different Levels of X-Irradiation (320 r and 2,560 r). *Department of Genetics, Iowa State College, Ames, Iowa, U.S.A.*

The pelvic regions of 58-day-old male mice of strains BALB/Gw and S were exposed to 320 or 2,560 r, the rest of the body being shielded. Animals were killed at 1, 8, 24 hours and at intervals up to 28 days after irradiation.

Except for dividing spermatogonia, exposure to 320 r induces little change in cellular composition until 1 to 3 days after irradiation during which time resting spermatogonia decline to less than 5% of control levels. Regeneration, apparent at 10 days, is well under way by 16 days. Dividing spermatogonia decline rapidly during the first 8 hours, increase slightly by 24 hours and decline further by 3 days. Later behaviour of the cells is essentially identical with that of interphasic spermatogonia. Spermatogonial necrosis is slightly increased following 320 r, the peak value of 2.4x control being reached at 8 hours.

Following 2,560 r, a significant decrease in frequency of resting spermatogonia is evident by 8 hours. By 5 days the level has declined to less than 10% of controls. Despite slight increases in frequency during the 5–10 day period no spermatogonia were present at 16 days. Dividing spermatogonia disappear rapidly during the first 24 hours and are absent at 5 days. No regeneration is evident at 16 days. Necrosis reaches a peak of 15% by 8 hours, but declines to zero by 24 hours. This is about 4 times the control level or almost twice the 320 r level.

These data support the conclusion that radiation-induced inhibition of chromosomal reduplication plays a prominent role in the initiation of the observed cellular changes. Also that radiation-induced cell death is more important at higher dosage levels. (This work has received assistance from Contract No. AT(11–1)107 from the Atomic Energy Commission.)

BUETTNER-JANUSCH, JOHN, S. J. BERHMANN, and F. P. THIEME. ABO(H) and Secretor Phenotypes and Human Fertility. *Laboratory of Physical Anthropology, University of Michigan, Ann Arbor, Mich., U.S.A.*

In recent years more and more evidence of the effect of the ABO(H) alleles on pregnancy outcome in certain types of matings has been presented. The secretor

gene apparently controls the presence or absence of water-soluble ABO(H) antigens in the tissues, and the role of this gene in abortion and infertility is under investigation in several laboratories. McNeil and his co-workers, for example, presented data which bear on the problem of early abortion, ABO(H) incompatibility and the secretor gene. The present paper will be a summary of some studies of the ABO(H) and secretor alleles among couples who are apparently unable to conceive. A report of the following studies on such couples, deemed infertile for no apparent reason, will be presented: (1) the determination of the presence or absence of the ABO(H) antigens in the saliva; (2) the determination of the presence or absence of the ABO(H) antigens in the semen of the males; (3) the determination of the presence or absence of these antigens on the surface of spermatozoa.

A discussion of the data presented and the problem of hetero-specific ABO(H)-Secretor matings as a mechanism of natural selection against certain ABO(H) phenotypes concludes the paper. (These studies were supported, in part, by USPHS grant H-3098, and by a grant from the Josiah Macy Foundation.)

BURDICK, ALLAN B., and TERUMI MUKAI. *Experimental Consideration of the Heterozygous Genetic Effect of Low Doses of Irradiation on Viability in Drosophila melanogaster. Department of Biological Sciences, Purdue University, Lafayette, Indiana, U.S.A.*

There is much evidence to indicate that the homozygous state of most irradiation-induced mutations is deleterious with respect to viability. However, in random mating populations, the homozygous state of a deleterious mutation is not achieved very frequently unless the heterozygous state is associated with higher-than-normal viability. In addition, most of the deleterious alleles of a gene in a random mating population will exist in heterozygous condition. Therefore, in determining the genetic effect of irradiation on random mating populations, it is much more important to determine the heterozygous mutant effects since most of the population consequence will be manifest from this condition.

Using a CMI-produced homozygous line of *Drosophila melanogaster*, we irradiated sperm with 0, 75, 150, and 300r and CMI-extracted about 80 homozygous lines per treatment. These experimental lines were then crossed to the original line to produce irradiation heterozygotes which were allowed to compete with homozygotes of the original line.

The most significant observation made was that the genetic variances of all of the irradiated groups were highly significantly greater than those of the control, the comparison for the 75r group being 96 versus 265. The variance increase of experimental groups was homogeneous, that is, not marked by few discrete viability differences, but by many small differences.

These mutations are dominant in the sense that they produce a phenotypic effect in heterozygous condition. The large variance increases produced by these low doses of irradiation indicate that a great many more mutations were produced than would be expected from present estimates of the number of potentially mutable genes and their average mutation rate per roentgen unit. The mutations produced appear to be equally likely to increase or decrease viability when in heterozygous condition.

BURDICK, ALLAN B. *See* MUKAI, TERUMI.

BURNHAM, C. R., E. TURCOTTE, and P. YAGYU. Interchanges Involving Three Chromosomes in Barley. *Institute of Agriculture, University of Minnesota, St. Paul, Minn., U.S.A.*

Among the intercrosses between the tester set of interchanges and unknowns there were two instances with a ⊙8. When first established, these unknowns had a ⊙4. In the test crosses of both parents, that with the unknown had a ⊙6, that with the other had a ⊙4. Test crosses have established one unknown as having chromosomes a, c, d interchanged.

The F_2 progeny of one of the intercrosses with a ⊙8 included 20 plants with a ⊙8, 9 with 7II and one with a ⊙4. The latter is a possible crossover in a differential segment.

BURTON, GLENN W. Genetic Variances in Pearl Millet (*Pennisetum glaucum*) and Their Significance to the Forage Breeder. *U.S. Department of Agriculture, Georgia Coastal Plain Experiment Station, Tifton, Ga., U.S.A.*

Since 1937, the U.S. Department of Agriculture and the Georgia Coastal Plain Experiment Station have been actively engaged at Tifton, Georgia, in the genetic improvement of Pearl millet, the most important annual pasture and forage grass in the southeastern United States. The protogenous flowering habit of this species has facilitated a breeding program similar in many respects to that followed by U.S. corn breeders. The development and evaluation of inbred lines from local and introduced varieties have been continued throughout the program.

Over the past ten years, 674 Pearl millet singlecrosses involving 248 well-established inbred lines have been compared in 15 forage yield tests designed to permit the estimation of additive and non-additive genetic variances. Some sets of inbreds have exhibited little non-additive variance. In other sets, most of the genetic variance has been of the non-additive type. On the average, 54.25 per cent of the total genetic variance for forage yield has been non-additive. These results indicate that: (1) Some increase in forage yield should result from the use of straight selection methods, (2) the maximum yield advance will be dependent upon the commercial use of the F_1 hybrid, and (3) tester crosses should be superior to topcrosses in evaluating new inbred lines.

Gahi-1, developed by using the procedures listed above, is an F_1 hybrid officially released for farm use in February 1958. In Regional Tests, it has out-yielded the common check by as much as 50%.

BUSS, KEEN. *See* WRIGHT, JAMES E.

BUTLER, LEONARD. Heterogeneity of Recombination Values in the Tomato. *Department of Zoology, University of Toronto, Toronto, Canada.*

Conflicting reports in the literature of recombination values that differ by as much as 15 $\pm$ 2%, has led to the investigation of the effect of the following

factors on crossing over: differential germination, misclassification because of genic interaction, genotypic background, coupling versus the repulsion phase, age of seed, temperatures during F_2 seed formation, and acidity of the soil in which F_1 plants are grown. In most cases where the recombination data are derived from more than five plantings, significant heterogeneity is shown. Differential germination accounts for most of the heterogeneity for crosses involving the genes *Xa*, *Wo*, *ro*, *rv* and *yv*. Misclassification of some genic combinations of *d*, *nc*, *br*, *dv*, *s*, *bk*, *tf*, and *H*, also account for some heterogeneity. Suggestive findings indicate that the recombination values of *wt* are influenced by the genetic background. Elimination of all other factors left no clear evidence that coupling data give different values from recombination data. Age of seed had some effect through its association with differential germination. The following data indicate that an environmental factor, probably temperature, has a major effect on recombination as shown by these values for different plantings of the same cross: 2,792 plants from seed grown in winter greenhouse gave 27.7 $\pm$ 1.72%, 1,755 plants from seed grown in the field gave 18.1 $\pm$ 2.29%, 1,565 plants from seed grown in summer greenhouse gave 11.3 $\pm$ 2.49%. Preliminary results indicate that recombination values are higher when F_1 plants are grown in acid soil. These findings indicate that data for linkage should not be indiscriminately combined, but should first be checked for heterogeneity.

CALEF, ENRICO. Integrated Genetics of Lambda Prophage and Coli K12. *Istituto di Genetica, Universita di Pavia, Pavia, Italy.*

The prophage genome is known to be attached to the genome of the bacterial host. Crosses between K12 derivatives, carrying marked lambda prophages, were carried out to find out more about the relationships of the two genomes.

Two types of crosses were attempted; the first type making use of Hfr1 and Hf2. Among the progenies selection was made for prototroph Gal + recombinants. Hfr1 on Hfr2 in these crosses gave the same type of results. The data might be summarized as follows: The determinants of the prophage are linked to the Gal locus in the following order: St. . . . . h co Gal Hfr1 T6. The test of linearity on these data shows apparent excess of exchange between St and h; the rate of exchange in that region ranging from 70 to 84%. This feature might be explained as the result of the recombination in incomplete zygotes. It is possible to test this explanation selecting St^r from the donor or some other marker linked to it. This new type of selection also requires the use of two pseudo-alleles Gal— and a donor Met— .The recombinants were selected to carry the following characteristics Met + from the F—; St^r from the Hfr, Gal + 54–2 for F— and Gal + 32–2 from the Hfr.

The preliminary results are promising in so far as they show no "recombination fraction" higher than 50%.

Owing to the peculiarity of the K12 mating system, this type of selection is somewhat inconvenient. Indeed, the conditions suitable for selection of St^r from Hfr favor reiterated mating. We are, therefore, studying a new Hfr able to transfer this particular region at high frequency and crosses in which Arginine + is selected from the Hfr instead of St^r.

CÂMARA, A., D. CASTRO, and M. NORONNA-WAGNER. Cytogenetics of Accessory Chromosomes in *Luzula campestris* DC. *Estação Agronomica Nacional, Sacavem, Portugal.*

Meiotic behaviour and inheritance of the accessory chromosomes have been studied in *Luzula campestris* DC. Results of selfed plants with one accessory chromosome as well as inheritance in reciprocal crosses, involving a plant with one accessory chromosome and a plant without accessories, are presented.

Accessory pairing in plants with two accessory chromosomes and their segregation to tetrads of pollen grains were studied.

CAMERON, DONALD R. Alien Substitution of a Locus Effecting Immunity to Blackshank in *Nicotiana tabacum*. *Department of Genetics, University of California, Berkeley, Calif., U.S.A.*

To maintain adequate fertility, sesquidiploid hybrids between *N. tabacum* and *N. plumbaginifolia* (*tbc-tbc-pbg*) and backcross derivatives were used in transferring a segment of *pbg* chromosome to a commercial type of *N. tabacum*. Backcrossing to diploid *N. tabacum* accompanied by selection for immunity leads rapidly to the establishment of a 24″+1′ condition. Such plants proved to be 85 per cent pollen sterile and high male transmission of the *pbg* chromosome showed that 25 chromosome pollen grains were functioning almost exclusively. The factor responsible for pollen abortion was separated from the dominant Blackshank locus (*Bs*) in three lines, two of which involved fragment chromosomes. The other included a fertile plant with the constitution 23″+3, the trivalent indicating that an exchange between *tbc* and *pbg* chromosomes had occurred. On selfing, populations were obtained exhibiting full immunity to the disease. Transmission percentages of about 40, 50 and 78, for ovule, pollen and selfing respectively, were in fair agreement with expectation if segmental substitution had been achieved. The corresponding transmission rates in 24″+1′ lines were roughly 9, 92 and 89%. Incorporation of other dominant genes from *pbg* has resulted in variegation in each instance. Even after the 24″ condition was reached progenies continued to segregate. The precise mechanism of this instability is as yet unknown. The low female transmission of the *Bs* chromosome in 24″ lines and the observed segregation from plants heterozygous for the *Bs* locus suggest that isolation of a satisfactory introgression product by this method may be more difficult than expected.

CAMERON, J. W., and D. COLE. Use of the Genes su_2 and *du* to Reduce Starchiness during Ontogeny in the Maize Kernel. *University of California Citrus Experiment Station, Riverside, Calif., U.S.A.*

In developing kernels, the recessive gene su_1 conditions occurrence of more sugar, less starch and much more water-soluble polysaccharide (WSP) than occur in the presence of its normal allel Su_1. Certain other genes, i.e., bt_1 and bt_2, also reduce starch and increase sugar, but do not cause an accumulation of WSP. The separate effects of the genes su_2 and *du* have been measured in several inbred backgrounds

in which *su*$_1$ is also homozygous. The most consistent effect of each gene is to slow the accumulation of starch, often beginning at 16 days after pollination or earlier. Starch was from 25 to 50% lower in homozygous *su*$_1$ *su*$_2$ and *su*$_1$ *du* than in *su*$_1$ on 6 tested backgrounds at 16 to 22 days. On 2 backgrounds there was no apparent difference among genetypes at 16 and 19 days. Absolute differences in starch were relatively independent of dry weight per kernel, within inbreds. Sugar and WSP content were not consistently different among the 3 genotypes, but increased tenderness and a less starchy taste were characteristic especially of *su*$_1$ *su*$_2$. Other changes in carbohydrate characteristics, such as the amylose-amylopectin ratio or the nature of the WSP fraction, may contribute to the difference in taste. Preliminary assays indicated little difference in rate of change of sugars and WSP during storage of kernels on the cob, but percentage increase in starch was usually lower in *su*$_1$ *su*$_2$ and *su*$_1$ *du* than in *su*$_1$. Incidence of mold on mature ears of *su*$_1$ *su*$_2$ and *su*$_1$ *du* was not significantly greater than on *su*$_1$ ears unless earworm had been present.

CAMPBELL, A. B. *See* MCGINNIS, R. C.

CAMPBELL, IAN. An Analysis of the Genetic Determination of Egg Weight in the Genus *Choristoneura* Led. (Lepidoptera: Tortricidae). *Forest Insect Laboratory, Sault Ste. Marie, Ontario, Canada.*

The genetic determination of egg weight in the genus *Choristoneura* was analysed on the basis of the egg weights of two geographic races of *C. fumiferana* Clem. (the eastern spruce budworm (B) and the western one-year-cycle spruce budworm (D)), the jack-pine budworm, *C. pinus* Free. (J), and their reciprocal F_1 hybrids. The differences between the egg weights of these different groups reflected genetic differences that were transmitted autosomally and x-chromosomally, as shown by the characteristic differences between reciprocal crosses. By measuring the differences between the egg weights of each parental type and all possible F_1 combinations, where the genetic constituent is fully known, it was possible to characterize the genetic effects of each specific x-chromosome and set of autosomes.

The results of this analysis indicated that the genetic units which determine egg weight act by controlling the rate of metabolic processes involved in egg growth, and that each unit acted by raising the rate of these processes by a specific power. The numerical value of effective genetic units on the autosomes of B, D, and J were 4, 10, and 5, and on the x-chromosomes, 22, 12, and 0 respectively.

CAPINPIN, JOSÉ, M., and UDHAI CHANPHAKA. Mutagenic Effects of Phytoextracts from *Antiaris toxicaria* (Pers.) Lesch and *Dioscorea hispida* Dennst. upon Living Plant Tissue. *College of Agriculture and Central Experiment Station, University of the Philippines, Los Baños, Laguna, Philippines.*

Previous studies on Philippine species of poisonous plants (Capinpin *et al.*, 1944, 1949, 1953) have shown that extracts from the bark of arrow-poison plant, *Lophopetalum toxicum* Loher, and root extract from a dog-poison plant, *Rourea erecta* (Blanco), Merr., were polyploidizing or growth-inhibiting or growth-pro-

moting agents. The present investigation deals with the action of the phytoextracts from *Antiaris toxicaria* (Pers.) Lesch, a source of powerful heart poison, and *Dioscorea hispida* Dennst., a source of arrow or spear poison, on living plant tissue.

The extracts were prepared in a specific manner so that the effectiveness of the toxic derivatives in the extracts simulates their quality as they occur in nature. These two extracts were tried at various concentrations on the plant testers, viz. root tips of onion (*Allium cepa* L.), seeds of mungo (*Phaseolus aureus* Roxb.), and seeds of soybean (*Glycine max* (L.) Merr.).

The extract from *Antiaris toxicaria* at one-half concentration (one part of the extract to one part water) and at one-fourth concentration (one part of the extract to three parts water) caused c-tumours in root-tips of onion when soaked for four hours and eight hours, respectively. The germinative ability of soybean and mungo seeds was delayed and sometimes inhibited if the periods of soaking were prolonged. C-mitotic figures in the cells of root-tips of onion occurred when treated with a right concentration and for a certain period of time.

C-mitotic figures were observed in the cells of root-tips of onion (at one-half concentration), similar to those produced by the extract from *Antiaris toxicaria* of the same concentration.

The results of the experiment appear to bring to light two naturally occurring substances capable of inducing cellular abnormalities and observable developmental variations.

CARLSON, ELOF AXEL. *Some Problems Involving the Cis-trans Effect in Drosophila. Indiana University, Bloomington, Ind., U.S.A.*

In the dumpy (dp) series of *Drosophila melanogaster*, four subloci are demonstrable by crossover tests involving outside markers and two others are suggested by differences in crossover frequencies. Omitting the gene-nest symbol dp, the indicated sequence is ′ol ′olv ′o ′lv ′ov ′v, where o, l, and v, respectively, denote the production of "oblique" wings, recessive lethality, and thoracic vortices. In heterozygotes, those mutant phenotypes (o, l, or v) are expressed which have genetic elements for them present in both chromosomes. Thus the *trans* heterozygote ′o/′lv is wild type, despite nearness of these subloci, whereas, ′ov/′ol and ′ov/′olv show very strong mutant phenotypes, although involving more distant subloci. The *cis-trans* effect of the pseudoalleles in the nest is just as extreme for the o, l, and v effects as the effect expressed in true homozygous alleles. Certain position effects can be shown in the nest by means of the specific modifier, Moiré. All members of the nest except ′v, show an oblique effect in the heterozygote when Moiré is present. No phenotypic difference is seen between the *cis* and *trans* arrangement of ′o and ′v or of ′ol and ′v. In the *trans* state however, ′o and ′lv (which are near together) show a more extreme effect in the wing than in the *cis* state. Heterochromatic position effects, found in rearrangements having one break near, or perhaps in, the dumpy-nest and one in heterochromatin, show "variegated" expression of the o and v effects and are suppressed by extra heterochromatin, which suggests a spreading effect throughout the nest. It is believed that a reinterpretation of the mechanism of the *cis-trans* effect is needed.

(This work was supported by pre-doctoral fellowships from the NSF (1955–56) and NIH (1956–57) and by a grant to Dr. H. J. Muller and associates from the U.S. Atomic Energy Commission, Contract AT (11–1)–195.)

CARSON, GWENETH L., and GERALD BRAVER. Studies of the Spouse Effect on Spontaneous and X-Ray Induced Lethal Mutations in *Drosophila melanogaster*. *Department of Zoology and Radiation Laboratory, University of California, Berkeley, Calif., U.S.A.*

Hildreth and Carson (1957) have shown that the frequency of spontaneous lethals in X-chromosomes derived from wild-type males was influenced by the type of female (Basc or Bv) that had been inseminated. This phenomenon, called the "spouse effect," is presently being studied along the following lines. (1) To determine if differences in the genetic backgrounds of the males examined for lethality were involved in the spouse effect, the genetic background of the Hildreth and Carson Bv (high mutation frequency line) lethals was replaced by one closely corresponding to that of the Basc (low frequency) lethals. If some of the Bv X-chromosomes were classified as lethal because of interaction with the remainder of the Bv genotype, but would not have been classified as lethal had they occurred in crosses with Basc, this test might result in reverted, non-lethal, wild-type males. At this writing, 31 of the 32 Bv lethals have been tested; all remain lethal in the Basc background. These differences, then, appear to be eliminated as a major component of the spouse effect. (2) Tests were made for the presence of a spouse effect on X-ray induced X-chromosome lethal mutations, using the Basc and Bv lines that had been utilized in the original investigation of spontaneous lethals. No clear-cut spouse effect could be demonstrated. (3) In order to study this problem further, a new experimental design is being used. The X-chromosomes of males coming from a highly inbred line are being tested for spontaneous and X-ray induced lethal mutations concurrently. One type of male and three types of females differing in genic and chromosomal constitution are being used. (This work was performed under the auspices of the Atomic Energy Commission.)

CARR, A. J. H. *See* OLIVE, LINDSAY S.

CARSON, HAMPTON L. Increase of Fitness in Experimental Populations following Introduction of One Haploid Set of Autosomes. *Washington University, St. Louis, Missouri, U.S.A.*

Four closed populations of the multiple third chromosome stock se ss k e^s ro of *Drosophila melanogaster* were allowed to rise to an approximately equilibrated size in small population chambers (Carson, Rec. Gen. Soc. Amer. *26*: 363). Food, temperature, light and space were kept constant. The upper asymptote of the postulated logistic curve is replaced by relatively minor oscillations of population size. After achievement of approximately stable age-distribution and sustained equilibrium size, one foreign adult male fly was introduced into each of two of the populations. The other two populations were continued as controls. The two males were siblings and were F_1s from a cross of a se ss k e^s ro female from the population by a + male (Oregon R). The foreign genetic material introduced into each population, therefore, consisted of one haploid set of + autosomes, in addition to a Y-chromosome, from the Oregon stock. The frequency of the wild-type phenotype increased with dramatic suddenness in three generations to about 90% in both of the experimental populations. Free recombination of the third chromosome genes is observed. Concomitant with this change, the population

size (in mg. wet weight) is approximately doubled. The biomass produced (wet weight of hatching adults/week) also abruptly rises. Data obtained to date indicate that both of these new attributes are maintained in the experimentals; the rise in fitness, therefore, appears to be permanent. In several pilot experiments, none of the mutant genes (se ss ro only in this case) has been completely eliminated by natural selection, even several years after return to mass culture. This fact, in addition to the initial speed of the change, suggests that the increased fitness is due to heterosis.

CASE, MARY E. Linear Relationships of Allelic Mutants at the *Pan-2* Locus in *Neurospora crassa*. *Botany Department, Yale University, New Haven, Conn., U.S.A.*

A new group of 37 allelic, pantothenic acid-requiring mutants at the *pan-2* locus in *Neurospora crassa* have been located in linkage group VI and show the following order and crossover values with markers in that group: *ylo* 1.7 *ad-1* 0.2 centromere 1.7 *pan-2* 7.7 *tryp-2*. Nine of these mutants, although blocked in the same step in pantothenic acid synthesis—the conversion of keto-valine to keto-pantoic acid—complement in certain combinations to form pantothenic acid-independent heterocaryons. These results provide evidence for two distinct functional units at the *pan-2* locus. An extensive tetrad analysis from a marked cross between two of these complementing mutants (*B5* and *B3*) indicates that the two functional units can also be separated genetically since asci have been obtained containing a pan prototroph of one cross-over type and a double mutant (*B5 B3*) of the reciprocal cross-over type. The composition of certain other asci indicates that mechanisms other than conventional crossing-over are probably also involved in the origin of some prototrophs. Despite this fact, the relative frequencies in the two possible cross-over categories of pan prototrophs from random ascospore platings of *B5* × *B3* establish an order of the mutants in agreement with the results from tetrad analysis, *B5* being proximal to *B3*. Similar random ascospore platings from crosses of all the remaining mutants to *B3* and *B5* indicate that non-complementing as well as complementing mutants can be grouped with either *B5* or *B3* on the basis of both the absolute frequencies of pan prototrophs and their relative distributions in the two cross-over categories. Additional crosses between single pan mutants, as well as crosses of single with double mutants indicate that recombination also occurs between mutants within each of the two groups. These data provide evidence for a linear relationship of mutants within each group. (Supported in part under a contract AT(30–1)–872 with the U.S. Atomic Energy Commission.)

CASPAR, ALAN L. *See* SINGLETON, W. RALPH.

CASPARI, ERNST. The Genic Control of Riboflavin in the Testis of *Ephestia*. *Wesleyan University, Middletown, Conn., U.S.A.*

In the testis sheath of wild-type *Ephestia,* a yellow pigment appears in the prepupa which has been identified as riboflavin. It can be determined micro-

biologically. The riboflavin content of the testis reaches a plateau in the early pupa, amounting to over 50% of the total riboflavin, and drops in the late pupa, the riboflavin being transferred to the Malpighian tubules.

The gene *a* causes an increased level of riboflavin at the plateau and an earlier drop than a^+. The gene *wa* inhibits the appearance of riboflavin in the testis sheath at any time. Three possible mechanisms for the inhibition of testis riboflavin by the gene *wa* were considered: (1) inability to synthesize riboflavin; (2) inability to accumulate riboflavin in the testis; (3) destruction of riboflavin.

The larva stops feeding in the middle of the last larval instar. If riboflavin was synthesized at the time of its appearance in the testis, an increase in the total riboflavin in wa^+ prepupae would be expected. Total riboflavin increases in both wa^+ and *wa* animals during the first half of the last larval period, and reaches its highest level in the early prepupa, i.e. before the accumulation in the testis is completed. The total riboflavin content of *wa* animals is at all times significantly lower than that of wa^+ animals. If *wa* testes are transplanted into wa^+ larvae, they do not accumulate riboflavin.

These results exclude possibility (1) since they suggest that no riboflavin is synthesized in wa^+ animals during the time when it appears in the testis sheath. Possibility (2) seems unlikely since in this case the riboflavin content of *wa* animals would be expected to equal that of wa^+ animals without being accumulated in the testis. The evidence is compatible with possibility (3) though additional hypotheses cannot be discounted. (Supported by National Science Foundation Grant G4495.)

CASTIGLIONI, M. C. The Behaviour of the Lymph Gland and the Migrant Haemolymph Cells, in Different Genotypes of *Drosophila melanogaster*. *Istituto di Genetica, Università di Milano, Milan, Italy.*

The morphology and the structure of the lymph gland have been studied during the third instar of *Drosophila melanogaster*, and differences have been found, which seem to be controlled by all three major chromosome pairs. The migrant cells of the haemolymph have been also investigated, and found classifiable into four types. The frequency of these cell types is also controlled by the genotype. A peculiar type of large cell proves to be the only one capable of producing melanin, after undergoing morphological changes: melanization (bringing about the production of melanotic masses) is controlled by recessive genes (tu) located—in the stocks investigated so far—in the second chromosome.

These findings contribute to the knowledge of the genotypic control of cell differentiation.

CASTRO, D. *See* CÂMARA, A.

CASTRO, L. E. *See* CAVALCANTI, A. G. LAGDEN, *et al.*

Cavalcanti, A. G. Lagden, D. N. Falcao, and L. E. Castro. The Interaction of Nuclear and Cytoplasmic Factors in the Inheritance of the "Sex-Ratio" Character in *Drosophila prosaltans*. *Centro de Pesquisas de Genetica, Universidade do Brasil, Rio de Janeiro, Brazil.*

The paper reports a phenomenon of "sex-ratio" in *Drosophila prosaltans*. This condition is found in natural populations and is evidenced by the existence of special females which give rise to exclusively or mainly female progenies.

A working hypothesis is presented, interpreting the results as due to an interaction between nuclear genes and cytoplasmic factors: *SR*—"sex-ratio" gene for unisexual female progenies; *sr*—recessive allele causing the appearance of males; *o'* (omikron)—plasmagenes necessary for the "sex-ratio" action. The "sex-ratio" females carry the plasmagenes omikron. The absence of males in their progenies is due to the death of the *XY* zygotes with an omikron plasmon. Normal males and females do not have the plasmagene. It is transmitted maternally. The reproduction of the plasmagene occurs only in the presence of the *SR* gene. In recessive homozygotes *sr/sr* the plasmagene stops its multiplication and gets lost. The only known function of the gene *SR* is to permit the reproduction of the plasmagene. It does not have any visible effects either in sex-ratio or in non-sex-ratio flies. The inbreeding of normal flies leads to the establishment of two kinds of homozygous stocks: the sex-ratio maintainer stocks *SR/SR* and the sex-ratio disrupter stocks *sr/sr*. The authors tested the hypothesis by backcrossing for two consecutive generations sex-ratio females homozygous for the *SR* gene to males of these two kinds of stocks. In the first case, only female progenies must hatch in both generations; in the second only females in F_1 and males and females in F_2 and subsequent generations. The results fully confirmed the expectation. The authors make a comparison between the "sex-ratio" trait in *Drosophila* and the "killer" character in *Paramecium*, pointing out the remarkable parallelism.

(This work was performed with funds of the Conselho Nacional de Pesquisas, Universidade do Brasil and the Rockefeller Foundation.)

Chai, Chen K. Isolation of Genetic Factors Affecting Body Size in Mice. *Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.*

A crossbreeding experiment using Large (mean body weight 36.6 gm.) and Small (mean body weight 14.6 gm.) strains of mice to produce F_1, F_2 and several backcross generations has been carried out. Results based on the earlier generations have indicated segregation of genetic factors responsible for difference in body size between these two strains and some degree of dominance of plus factors over minus factors. A minimum of 13 segregating units was estimated. In an attempt to isolate these units, repeated backcrossing to the Large and Small mice along with mass selection for large and small body size has been continued. Selection began at the second reciprocal backcross generations. The Large mice have a slower turn-over rate in reproduction and the backcrossing to the Large is at the fourth and fifth generations, while backcrossing to the Small mice has completed the sixth generation. Only the results of the Small mice and the backcrosses to the Small are given at present, using 60-day body weight as the index of body size, and including a total of 1,816 mice.

The Small mice produced during the years 1953 and 1954 were contemporary with the F_1 and the first backcross (B_{1S}) generations, in the years 1955 and 1956 with B_{2S}, B_{3S} and B_{4S}, and in the year 1957 with B_{5S} and B_{6S}. The mean body weights and variances within litters of the Small mice and the backcrosses will be presented. Beginning from the fourth generation the backcrosses selected for large body size showed no more reduction in mean body weight and those selected for small body size approached the mean weight of the Small strain. The variances were in general as expected except those of $B_{5S}(L)$ and $B_{6S}(L)$ which appeared smaller than expected.

There was a slight increase in body size of the Small mice. The Large mice do not have minus factors which are not present in the Small mice. It is likely that single factor has been isolated in the $B_{4S}(L)$ to $B_{6S}(L)$ generations, which causes an increment of body weight of the Small mice by approximately one gram. If more than one plus factor have been introduced from the Large to the Small mice, only one of them would be present in any individual mouse of these generations. (Aided by grant C-3108 from the National Cancer Institute of NIH, U.S.A.)

CHANPHAKA, UDHAI. *See* CANINPIN, JOSÉ M.

CHASE, SHERRET, S. The Utilization of Parthenogenesis in Maize Breeding. *DeKalb Agricultural Association, Inc., DeKalb, Ill., U.S.A.*

During the past few years several thousand parthenogenetically derived monoploid maize plants have been isolated. From these plants, through self-pollinations of the occasional, partially fertile individuals several hundred homozygous diploid progeny have been developed. In tests of these as parents of commercial type hybrids, a high percentage have proven to be superior in performance. This result has encouraged the continued use of the "monoploid method" as a practical tool for maize breeding.

CHENNAVEERAIAH, M. S. *Campelia zanonia*: Is It a Polyploid? *Institut Botanique, Montreal, Canada.*

The role of polyploidy as a potent factor in the evolution of plant species, especially among the flowering plants, is well known. It is true that up to 30–35% of the Angiosperms are polyploids and there is a growing tendency among botanists to list more and more of these as polyploids of one kind or the other. The Tradescantias alone are known to include auto-, allo-, aneu- and segmental-polyploids. The question is whether *Campelia zanonia* (L.) H.B.K., a monotypic genus of the tribe Tradescantieae-Hexandrae with chromosome number $2n = 16$, suggested to be a tetraploid with basic chromosome number of 4 (Darlington, J. Genetics *35*: 259–280), is a polyploid at all. This suggestion of Darlington is based on the observations of Anderson and Sax (Bot. Gaz. *97*: 433–476) who reported associations of 4 at meiosis.

Since *Campelia zanonia* is being grown at the Montreal Botanical Gardens, it was thought worthwhile to investigate its suspected polyploid nature. If it is a polyploid, it should be an autotetraploid as it is monotypic. Autopolyploids in nature, however, are very rare except for plants such as *Galax aphylla* which is an autotetraploid (Baldwin, J. Heredity *32*: 249–254) and *Tradescantia bracteata* which is an autotriploid (King, J. Heredity *24*: 253–256).

Karyomorphological studies performed on *Campelia zanonia* reveal that there are only two genomic sets of chromosomes.

Configurations of meiotic chromosomes in a hundred or more microspore mother cells showed complete absence of multivalents. The association of four observed by Anderson and Sax was probably due to overlapping and the mention of four bivalents in it may be a misprint. Since no taxon of *Campelia zanonia* with chromosomes $2n = 8$ has been discovered (as was found by Baldwin in *Galax aphylla,* diploid with $2n = 12$ and tetraploid with $2n = 24$ chromosomes), and since the present cytological studies reveal only two genomic sets of chromosomes and the absence of multivalent formation, *Campelia zanonia* ($2n = 16$) should be regarded only as a diploid on the criterion of chromosome number.

CHILDERS, W. R. A Case of Complete Male Sterility in *Medicago sativa* L. *Forage Crops Division, Central Experimental Farm, Ottawa, Canada.*

A completely male sterile plant of *Medicago falcata* × *M. sativa* origin was obtained from Dr. V. C. Brink of the University of British Columbia. A cross was made with a male fertile plant and cytological and genetic studies were made of the male sterile condition.

The course of microsporogenesis of the male sterile plant was regular until after the tetrads of spores were formed. Then rapid degeneration of the developing spores occurred, a condition which was accompanied by the appearance of extremely dense, swollen tapetal tissue. No mature pollen grains were observed. In the F_1 generation all variates were male fertile. When F_2 and backcross families were grown and observed, 63:1 and 15:1 ratios of fertile-sterile variates were obtained in the former and 7:1 and 3:1 ratios in the latter families. Thirty-four F_2 plants, selected from a population segregating 15:1 for the male-fertile and male-sterile characters, were selfed. F_3 families were grown out and observed for male fertility. These families were grouped into three classes, non-segregating, segregating 15:1 and segregating 3:1 in a proportion of 7:4:4 respectively. These results supported the 15:1 ratio obtained in the F_2 family. Considering the F_2 and F_3 results, it was concluded that complete male sterility was determined by three recessive genes. The symbols proposed for these genes were *ms*3, *ms*4 and *ms*5.

CHOVNICK, A. Structural and Functional Aspects of Pseudoallelism in *Drosophila melanogaster*. *University of Connecticut, Storrs, Conn., U.S.A.*

Recombination studies are reported between garnet mutants of *Drosophila melanogaster* (chromosome 1–44.4) in both free-x and attached-x heterozygous females. The pattern of recombination for closely linked markers in association

with the recovery of non-garnet chromosomes from mutant heterozygotes is consistent with a pseudoallelic organization in the order g^2 — g^{50e} — (g^3,g). The estimated recombination frequencies indicate that garnet is the tightest cluster of mutants thus far to yield to recombination in this organism. The linkage distances permit an estimate of the resolving power of recombination analysis in *Drosophila.* In addition to the recovery of non-garnet chromosomes associated with recombinations between the closely linked markers, there were recovered, from several heterozygous combinations, non-garnet chromosomes not associated with marker recombination. Further investigations reveal that (1) these "reversions" produce a phenotype indistinguishable from wild type; (2) the origin of the "reversions" does not involve suppressor mutation; (3) spontaneous reverse mutation is not the mode of origin of the "reversions."

The relationship between the results of this study, and the results obtained with reddish-alpha in *Drosophila virilis,* and cases of gene conversion seen in microorganisms, will be discussed.

CHOWN, BRUCE. *See* LEWIS, MARION.

CHU, ERNEST H. Y., and NORMAN H. GILES. Chromosome Complements and Nucleoli in Normal Human Somatic Cells in Culture. *Botany Department, Yale University, New Haven, Conn., U.S.A.*

Cytological studies have been made on human somatic cells in cultures derived from explantation of six surgical biopsies and of two fetuses (all from American Whites). In all eight individuals the diploid chromosome number was 46. In one human embryo the same number was found in cells from seven different organs. In another individual squash preparations of primary spermatocytes showed the same diploid number as testicular fibroblasts *in vitro.*

Detailed idiograms based on normal diploid cells *in vitro* from different human individuals have been constructed. By chromosome matching, the human Y-chromosome has been recognized. Further studies confirm and extend the observation by Tjio and Levan (1956) of satellites on human chromosomes and indicate that there are two pairs of homologues with satellites, both having submedian centromeres. One of these chromosomes is short (about 2μ) and the other medium (about 4μ) in length. In each chromosome, the satellite is located on the shorter arm.

Our studies also indicate that the human somatic interphase nucleus contains basically four spherical nucleoli which are in fact two pairs with approximate diameters of 1 and 2μ respectively. In certain cells, these four nucleoli are found to have fused into three, two or one units. All of the nine possible fusion patterns with the expected nucleolar volume relationships are found. The relationship between the satellited chromosomes and the number and structure of nucleoli will be discussed.

(Supported in part under a contract AT(30–1)–1908 with the U.S. Atomic Energy Commission.)

CHUNG, C. S., and N. E. MORTON. Discrimination of Genetic Entities in Muscular Dystrophy. *Department of Medical Genetics, University of Wisconsin, Madison, Wis., U.S.A.*

A study was made to separate genetic groups of muscular dystrophy by discriminant functions. Approximately 800 cases from Wisconsin and from comparable studies in the literature were classified on pedigree information into four non-overlapping groups: I, an affected parent; II, not I, but an affected girl in family; III, not I or II, but a maternal male relative affected; IV, not I, II, or III, only brothers affected. Twenty variables associated with mode of onset and clinical condition were used for the initial discrimination, with the addition of laboratory data and muscle function evaluations for the Wisconsin material.

The first three groups are differentiated by parental consanguinity, localization of first symptoms, pseudohypertrophy of calves, and course of the disease. When the relevant variables are combined into discriminant functions, there is good separation of I and III, but appreciable overlap between I and II and between II and III. Isolated probands of class II have significantly less parental consanguinity and higher average of age onset than familial probands. The data suggest that about 60% of class II cases are attributable to autosomal recessive genes and the remainder are sporadics of uncertain origin. No clinical difference was observed between sexes of familial cases of class II, whereas in class I the males have significantly later age of onset than females.

Using the discriminant between II and III, class IV shows a bimodal distribution, the modes of which approximate those of groups II and III. The discriminant permits phenotypic classification of IV cases into probably of genetic type II or probably of genetic type III, with a calculated error of about 15%.

Further investigations by the discriminant methods of Bartlett will be reported, together with linkage studies and evidence on the value of muscle function evaluations and laboratory tests in group discrimination. High levels of serum aldolase and transaminase are characteristic of sex-linked cases.

CHUNG, C. S. *See* MORTON, N. E.

CLARK, GORDON MURRAY. Chromatographic Studies on X-Radiated Protozoa and the Medium on which they have been growing. *Department of Zoology, University of Toronto, Toronto, Canada.*

The ciliate *Tetrahymena pyriformis* grows on a defined medium consisting of various amino acids, vitamins, nucleic acids, salts, etc. Strains TC 89, TC 105, TC 110, and TC 148 (variety nine) were X-radiated at dosages of 100,000 r to 600,000 r at 100,000 r intervals (5,000 r per minute at 25°C). The ciliate was washed in doubly distilled water and starved 24 hr. previous to irradiation. After irradiation approximately 25,000 organisms were placed in 5 cc. of defined medium and allowed to grow for 120 hours at 25°C (slanted tubes). Irradiated and non-irradiated organisms were also placed on 5 cc. distilled water for a period of 72 hours. Following the incubation period, samples of media and distilled water (0.05 cc.), with the ciliates removed were placed on chromatographic (What-

man #1) and a 30 × 30 cm. two-dimensional chromatogram was run using 80% aqueous phenol and butanol-acetic acids as solvents. A 0.2% solution of ninhydrin in water-saturated n-butanol was used as a developer. Studies with irradiated ciliates placed on distilled water indicated amino acid leakage takes place after irradiation. No such effect could be demonstrated in the non-irradiated controls. Comparative chromomatographic studies with the media on which irradiated and non-irradiated ciliates have been growing indicate that serine and perhaps methionine are utilized rapidly in the irradiated organism. At present nucleic acid and cell permeability studies are in progress.

CLARKE, C. A., and P. M. SHEPPARD. Disease in Relation to Human Polymorphic Systems. *Departments of Medicine and Zoology, University of Liverpool, Liverpool, England.*

Little is known of the factors which stabilise human polymorphisms, but three medical aspects of the problem have been studied. (1) *The ABO blood groups and gastro-intestinal disorders.* The association between peptic ulcer and blood group O was originally described by Aird and his colleagues in 1954. Family studies carried out by us where the unaffected sibs act as controls give no support to the hypothesis that group O predisposes to ulcer, whereas when general population controls are used the association has been found in many parts of the world. This apparent discrepancy will be discussed.

Non-secretion of the ABO blood groups and duodenal ulcer also appear to be associated, and possible explanations of this will be reviewed. Some light on the matter is obtained by estimating the fucose content of saliva, which gives a quantitative index of the amount of blood group substance present. Data on this matter will be considered.

Using fluorescine-labelled antibody technique, some information has also been obtained about ABO antigen sites on duodenal cells.

(2) *The ABO blood groups and Rhesus incompatibility.* It has been known since 1943 that incompatibility of ABO mating seems to confer a definite protection against haemolytic disease due to Rh incompatibility. "Rhesus" families have been studied to determine the ABO blood group and secretor character of children born before Rh antibodies have been produced in the mother, to test the hypothesis that the immunising foetus is usually ABO compatible.

(3) *The relationship between adenomatous goitre and the ability to taste PTC.* Earlier work has been confirmed which showed that there is a 40% incidence of non-tasters in patients with adenomatous goitre compared with 30% in a control series. These findings will be related to animal experiments in which water and PTC solution respectively have been offered to "taster" and "non-taster" rats.

CLARKE, C. A., and P. M. SHEPPARD. A Genetic Investigation of Mimicry. *University of Liverpool, Liverpool, England.*

Since 1862 much speculation on the mode of evolution of Batesian mimicry has occurred, but little experimental evidence has been accumulated. Two main hypotheses have been put forward. That subscribed to by R. C. Punnett and R. Goldschmidt maintains that, in butterfly mimicry associated with polymorphism, each new mimetic pattern must arise perfectly formed as the result of a single mutation,

since the differences between all forms within a population are controlled by single genes. In contrast R. A. Fisher and E. B. Ford maintain that a mutant giving some mimetic resemblance arises and the pattern is gradually improved by the selection of modifiers of the new mutant's effect.

We have shown that in the African mimetic butterfly *Papilio dardanus* the sympatric South African forms are controlled by a multiple allelomorphic series and that *hippocoonides* is recessive to *natalica* which is recessive to *cenea* which is recessive to both *leighi* and *trophonius*. *Leighi* and *trophonius* have an intermediate heterozygote called *salaami*; and *natalica* and *trophonius* also have an unnamed intermediate heterozygote.

If the gene controlling *cenea* is introduced into the gene-complex of *P. dardanus dardanus* from Ghana, where *cenea* itself does not occur, an imperfect mimetic resemblance results, demonstrating the presence of modifiers improving the mimicry in South Africa. However, when South African *cenea* is introduced into the gene-complex of *P. d. dardanus* from Uganda, where *cenea* is found at low frequency, there is only a slight decrease in the perfection of the mimicry. Two rather similar allopatric forms *hippocoonides* and *hippocoon*, which mimic different subspecies of one model, do not differ by a pair of allelomorphs as predicted by Goldschmidt but are multifactorially controlled as predicted by Ford. These results demonstrate the validity of Fisher and Ford's view on the evolution of mimicry. Racial crosses using the primitive non-mimetic subspecies from Madagascar are being made and these should throw further light on the evolution of mimicry.

CLAYBERG, CARL D. Genetically Determined Precocious Centromere Division in *Lycopersicon esculentum* Mill. *Connecticut Agricultural Experiment Station, New Haven, Conn., U.S.A.*

A meiotic abnormality found in an unfruitful mutant in the tomato variety Earlypak is controlled by a single recessive gene *pc*. The mutant is characterized by the regular occurrence of precocious centromere division of chromosomes in meiosis, resembling a case reported by Lamm (1944). The division begins in anaphase and is complete in practically all of the chromosomes by prophase of the second division. Following the separation of homologs, some chromosomes lag and frequently divide into daughter chromosomes during late anaphase. Most of the chromosome centromere division seems to occur during interkinesis. At metaphase of the second division all undivided and divided chromosomes *regularly* become oriented on the plates. Both types segregate to the poles, the latter without again dividing. As a consequence of considerable lagging in the second division, restitution nuclei are frequently formed. The resulting diploid gametes may give rise to polyploid offspring although the mutant normally possesses extremely low pollen and ovule fertility.

CLAYTON, GEORGE A. The Effect of Size of Breeding Population on Residual Genetic Variance. *Institute of Animal Genetics, University of Edinburgh, Edinburgh, Scotland.*

This experiment is part of a general investigation into the nature and magnitude of genetic variation retained in breeding populations. The aim was to measure the

actual residual genetic variance after a high theoretical level of inbreeding had been reached in populations inbred at different rates.

The character measured, the sum of the bristles on the 4th and 5th sternites of *Drosophila melanogaster*, has a total variance of 11.2 and an additive genetic variance of 5.8 in the large panmictic cage population which provided all the experimental lines. At least 80% of the total genetic variance is additive and the realized heritability is 50% $\pm$ 2%. The character appears to be affected by large numbers of genes of small effect and there appears to be little if any correlation between bristle number and fitness, at least within the phenotypic range encountered in the base population. This character thus appears to meet all the requirements of the theoretical drift model.

Four rates of inbreeding were studied: 20, 10, 5 and 1 pair of parents per generation. There were 4–5 replicates within each group. All lines were subjected to two-way selection for three generations when they had reached a computed level of inbreeding of 87%. The results showed that at the slower rate of inbreeding, viz., the 20's and the 10's, considerably more additive genetic variance was retained than theory would allow. The 5's and full-sibs declined according to prediction. There was a noticeable degree of heterogeneity between replicates.

The principal conclusions are that computed Coefficients of Inbreeding will almost invariably overestimate the rate of loss of genetic variance and that the effective number of parents must be extremely small for the drift process to play a significant role.

CLISE, RONALD L. Factors Contributing to the Low Survival Rate of Bar Eyed *Drosophila melanogaster*. *Fenn College, Cleveland, Ohio, U.S.A.*

Two populations of *Drosophila melanogaster* were grown in a series of culture bottles connected with each other through large diameter glass "T" tubes. This method, an adaptation of the population bottles of Reed and Reed, allowed relatively large population to be maintained in minimum incubator space without undue fluctuation in population size.

Flies taken from the population were used to determine mating capacities and the frequencies of the various mating combination, bar $\times$ bar, wild $\times$ wild, bar $\times$ wild, etc. In addition, the fertility of females was determined with regard to both the number of eggs laid and the number of imagos resulting.

The mating capacities of wild and bar eye males, based on 192 matings, were in the ratio of 1:.52. Fertility of females was in the ratio of 1:.94:.36 for heterozygous bar, wild, and homozygous bar females respectively, based on the number of eggs. These ratios were, in the same order, 1:.93:.46 when based on the number of imagos produced by 159 females. The fertility ratios were established after it had first been determined that the type of male involved in each mating did not significantly affect the fertility of the female.

The above ratios were expressed as selection factors and predictions of gene frequencies were made. After adjustment to account for the overlapping of generations inherent in sampling of populations, the predicted gene frequencies were not significantly different from the observed frequencies. Reduced mating capacity on the part of the male, and reduced fertility in the female thus appear to be the major factors contributing to the failure of the bar eye gene in competition with the wild type.

Cocito, Carlo, and Henry J. Vogel. Heritable Lowering of an Enzyme Level and Enzyme Repressibility Observed upon Continuous Cultivation of *Escherichia coli* in the Presence of a Repressor. *Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey, U.S.A.*

Escherichia coli (ATCC No. 9637) was grown at about 30°C, without forced aeration, in a continuous cultivation device, essentially the turbidostat of Bryson (Science, *116*: 48), on a glucose-salts (minimal) medium with or without a supplement of L-ornithine hydrochloride (20 mg. per liter). The resulting cultures were sampled at daily intervals and streaked to single colonies on unsupplemented solid medium. From such colonies, stock cultures in liquid minimal medium containing casein hydrolysate (0.2%) were derived. For assays of the enzyme acetylornithinase, inocula from each stock culture (after preliminary growth on liquid minimal medium) were cultivated on (*a*) minimal medium, and (*b*) minimal medium supplemented with L-arginine hydrochloride (0.1 micromole per ml.). Enzyme levels (in enzyme units per mg. protein) and enzyme repression were determined, as previously described (Vogel, The Chemical Basis of Heredity, Johns Hopkins, 1957, p. 276), in extracts of sonically disrupted organisms. Both ornithine and arginine are repressors that antagonize acetylornithinase synthesis.

Between the third and seventh day of continuous cultivation in the presence of ornithine, isolates were obtained that exhibited acetylornithinase levels(determined without arginine) ranging downward to 10–15% of those of the inoculum used. Some such isolates showed greatly lowered or no repressibility with respect to the synthesis of this enzyme. In contrast, isolates from continuous cultures grown without ornithine showed no substantial decreases in acetylornithinase levels, but often showed increases; the repressibility of these isolates tended to remain high.

The isolation of clones exhibiting reduced levels of acetylornithinase and reduced degrees of repressibility of acetylornithinase formation aids in interpreting the further finding that strains of several genera of Enterobacteriaceae showed considerable variation, regardless of taxonomic position, in acetylornithinase level and repressibility. (Aided by the Damon Runyon Memorial Fund and the National Science Foundation.)

Cockerham, C. C. *See* Legates, J. E., *et al.*; Robinson, H. F., *et al.*

Cockerham, C. Clark. *See* Kojima, Ken-ichi.

Coe, E. H., Jr. A Continuing, Non-Reverting Conversion-Type Phenomenon at the *B* Locus in Maize. *U.S. Department of Agriculture* and *University of Missouri, Columbia, Mo., U.S.A.*

The established alleles at the *B* locus are *B*, B^w, and *b*, in order of dominance and decreasing depth of color. Dominance is very nearly complete, and standard segregation ratios are obtained. In a progeny of self-pollinated *B B* (strong-colored) plants, two weak-colored individuals similar to B^w appeared. Self-pollinations of these exceptions and crosses to *B B* egg parents failed to give strong types in the progeny. Even after three cycles of selfing and of repeated backcrossing to *B B* male and female no strong plants have been obtained. The exceptional weak

type is designated *B′*. Crosses of *B′* × *b b* give weak (*B′ b*) and on selfing show 3 weak: 1 colorless. Crosses of *B′ b* × *B B* give half weak (*B′ B*) and half strong (*B b*). These results demonstrate the absence of a transmissible cytoplasmic factor and mark the effect as genetic and at the *B* locus. The effect is not due to exclusive transmission of the *B′* chromosome since normal segregation with *b* and linked markers is obtained. The effect differs from the phenomenon at the *R* locus, reported by Brink, in being entirely regular in the change which occurs, in continuing to "pass on" the effect, and in failing to revert to the standard type when made homozygous. Since the immediate generation from crosses is weak, whatever change occurs is assumed to come about at fertilization. *B′* plants continue to appear occasionally in the original *B B* line.

COHEN, CARL. A Second Interaction Antigen in the Rabbit. *Battelle Memorial Institute, Columbus, Ohio, U.S.A.*

The cellular antigen which results from the interaction of two allelic genes is relatively rare. Irwin has found a hybrid substance in a species cross in doves and Miller has shown that in at least one of these crosses the hybrid antigen is the result of interaction of two allelic genes, each of which is capable of giving rise to a detectable antigen. The interaction antigen *I* occurs in the heterozygote Hg^A/Hg^D of the three allele series Hg^A, Hg^F, and Hg^D. In a continued study of this locus a second interaction antigen has been found to occur in the heterozygote Hg^A/Hg^F. This antigen, labelled *J*, is most unusual in that it also occurs as the direct product of the gene Hg^D.

The antibody which detects this second interaction antigen was prepared by the immunization of recipient of type A by the blood of a donor of type D. By suitable absorption an antibody was isolated which agglutinates the cells of animals of genotype Hg^A/Hg^F and also animals of the genotype Hg^D. A second antibody prepared by absorption of the original whole serum agglutinates cells of type D only. A third antibody, called anti-K, was also prepared from the original serum and is capable of agglutinating the cells of all animals carrying the gene Hg^F or Hg^D.

Breeding experiments using matings of type A by type F yield offspring of type A F J. In no case does either parent react with anti-J; in every case the offspring reacted with anti-J.

The antigen J resulting from the interaction of the genes Hg^A/Hg^F appears to be serologically identical to the antigen J which is controlled by the gene Hg^D. Antigen K occurs as the result of the presence of either the gene Hg^D or the gene Hg^F.

COLE, KATHLEEN. A Cytogenetic Investigation of Some Marine Algae of the Pacific Coast. *Department of Biology and Botany, University of British Columbia, Vancouver, B.C., Canada.*

A cytogenetic study, based chiefly on marine algae grown in culture, is being carried out in an attempt to solve some of the problems concerning the speciation of certain members of the Pacific Northwest flora. Such a study establishes the presence or absence of morphological alternation of generations on a cytological basis. Species of several genera in each of the Chlorophyta, Phaeophyta, and

Rhodophyta have been observed, and developmental stages of some of these have been successfully obtained in culture. Belling's iron-aceto-carmine squash technique was used with good results on all forms so far investigated. Depending on the particular alga under study, certain modifications such as prefixation in different solutions, mordanting with iron alum, and prolonged heating were found necessary. This method is particularly suitable for a cytogenetic survey because it is simple, rapid, stains the nuclei effectively, and may be used even in the field. Flagella, cell walls, cytoplasmic inclusions, pyrenoids, nuclear membranes, nucleoli, and chromosomes were readily observed following treatment with iron-aceto-carmine. Unusual mitotic divisions similar to those reported in certain fungi were noted in several forms. The study is continuing in an attempt to obtain chromosome counts and a more detailed knowledge of both the cytological and morphological aspects of the life cycles of the marine algae of this region.

COLOMBO, GIUSEPPE. X-ray Induced Impairment of Fecundity and Fertility of *Bombyx mori* females. *Istituto di Zoologia dell'Università di Padova, Padova, Italy.*

Each female of the silkworm *Bombyx mori* L. lays all her eggs at one time; the steps of egg maturation are rather strictly related to the developmental stages. Therefore it is possible to irradiate at various stages females whose ovaries contain germ cells almost at the same stage. Females, as well as males, have been irradiated by X-rays (Therapeutic Philips Apparatus, 50 kv, 2mA, F 0.5 A1, d. f. 60 mm; 950 r/min.) at third and fifth larval stage and early and late pupae with 1,000 r, 2,000 r, 4,000 r and some with 8,000 r, by varying the length of exposure.

The irradiated females were crossed with untreated males of the same egg-brood, the males with untreated females of another egg-brood of the same race. The number of laid eggs (fecundity) and that of hatched larvae (fertility) were recorded.

Above 2,000 r fecundity decrease is higher among the females treated at the fifth larval stage and early pupae than among the irradiated late pupae. These effects show that the sensitivity of the oocytes is higher during the 2nd period of growth than during vitellogenesis. Fertility, which can be considered a test for induced dominant lethals, is more affected in later stages of oogenesis than in earlier ones, which means that metaphase chromosomes are more sensitive than prophasic ones. These results are in agreement with those obtained in *Drosophila.*

The impairment of fecundity appears to be mainly a cytoplasmic effect of irradiation which would affect the differentiation and growth of oocytes rather than the structure of chromosomes.

A stage of oocyte growth particularly sensitive to X-rays is the second period of growth when nurse cells produce large amounts of RNA which is used for the synthesis of structurally homogeneous cytoplasm in the oocytes. X-ray treatment probably interferes with RNA production and/or RNA utilization.

CONGER, A. D. The Fate of Metaphase Exchanges. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

Radiation-induced chromosomal damage is measured by observation of all aberrations at metaphase in favorable materials, and by observation of bridges and

fragments at anaphase in less favorable materials. The assumption is made that a constant proportion of the important exchange aberrations (dicentrics and rings) seen at metaphase give rise to bridges at anaphase. Sheldon Wolff has recently shown this is not true, at least in barley, where he found metaphase exchanges increased as dose^2, anaphase bridges as dose^1. Metaphase exchanges could fail to produce detectable bridges at anaphase either by (*a*) dividing in a "fall-free" manner, thus making no bridge, or (*b*) making a bridge, which then breaks.

It was possible to analyse in *Tradescantia* exchanges at metaphase, bridges and fragments at later anaphase, and also exchanges and their manner of separation (as fall-frees, criss-cross or interlocked bridges) in very early anaphase. With increasing dose, metaphase and early anaphase exchanges increased as dose$^{1.6}$, and were seen in almost equal numbers. Bridges at later anaphase increased as dose^1. Over the dose range studied, a constant proportion, about half, of all exchanges separated in a fall-free manner. The discrepancy between metaphase exchanges–detected anaphase bridges is therefore not caused by a changing fraction of exchanges that fall free, but must be caused by bridges that break, the fraction breaking increasing with dose. An association between restituted radiation-induced breaks, and breakage of anaphase bridges, could explain the results. Radiation-induced breaks increase proportionately to dose; if restituted breaks are weak points in a bridge, then breakage of anaphase bridges would likewise increase proportionately to dose.

CORCORAN, PATRICIA A. *See* ALLEN, FRED H., Jr., *et al.*

CORDEIRO, A. R., J. IVES TOWNSEND, J. A. PETERSEN, and EUTERPE C. JAEGER. Genetics of Southern Marginal Populations of *Drosphila willistoni*. *University of Rio Grande do Sul, Brazil*, and *University of Tennessee, Knoxville, Tenn., U.S.A.*

Drosophila willistoni, a neotropical species which prefers the warm humid rain forests of northern and central Brazil, has nevertheless a wide distribution ranging from southern Florida to Montes Tordillos, 200 km. south of Buenos Aires. For the purpose of investigating the genic polymorphism of marginal populations, strains in a sample from the "Hacienda El Destino," near the city of La Plata, were rendered homozygous for a *second* or a *third* chromosome. Of a total of 320 different homozygous second chromosomes, 6.25% were semilethal and 27.18 ± 2.3% were lethal, giving a combined value of 33.43 ± 2.6% lethals + semilethals. This value is significantly lower than 41.2 ± 1.1% for tropical Brazil determined by Pavan *et al.* (1951) in a total of 2,004 strains and does not differ from the 32.24 ± 1.6% Cordeiro *et al.* (1958) found in 794 strains from Rio Grande do Sul, Brazil, or from the 31.1 ± 4.4% found by Townsend (1952) in 109 strains from Florida. Of the 97 different homozygous third chromosomes, 20.61 ± 4.2% were lethal, and the combined value of 28.8 ± 4.6% lethals + semilethals does not differ significantly from the 31.1 ± 1.4% Pavan *et al.* (1951) found in 1,166 strains from tropical Brazil or the 32.8 ± 4.2% Townsend (1952) found in 122 strains from Florida. This work confirms the findings of Townsend for northern marginal populations of *D. willistoni* and supports the explanations given in Carson's (1955) review of the problems of genetic composition of marginal populations of several

species, according to which a lower degree of genetic polymorphism is correlated with harder environmental conditions, less specialization, and a smaller number of ecological niches occupied by the organism. (Work supported by grants from the Rockefeller Foundation, the Conselho Nacional de Pesquisas (Brazil), and CAPES (Brazil).)

CORDEIRO, A. R., F. M. SALZANO, V. B. MARQUES, and J. A. PETERSEN. Experimental Shift from Genetic Balance of Natural Populations of *Drosophila willistoni*. *Division of Genetics, University of Rio Grande do Sul, Porto Alegre, Brazil.*

Southern natural populations of *Drosophila willistoni* inhabit isolated small woods scattered in the grassland region of Tainhas in the north of Rio Grande do Sul. Samples of these populations were crossed with northern Brazilian races and the F_1 hybrids (N/S) proved to be heterozygous for six "new" inversions not present in the Tainhas region: XL-Bb, Dd, Ff, Gg, Hh; XR-Ee; and carried more than 50 per cent of IILAa IIR Ee and III Jj (chromosome map, Dobzhansky, 1950; Da Cunha *et al.*, 1950). More than 60,000 hybrids N/S individuals raised in the laboratory were released at each of the three isolated woods no. I, IV and V, at the beginning of the summer of 1956, when their population sizes are at least 30 times smaller than the hybrids received. Cytologic analysis of control and experimental woods was performed in eleven samples made during the summers of 1956 and 1957, totaling 698 karyotypes of single F_1 larvae produced by isolated cultures of one female each. Statistical analysis reveals heterogeneity between some inversions in control (1956, 1957), but it is more evident between control and experimental woods. It is of interest that three out of six "new" inversions were incorporated in wood I, but not one in IV and V. The incorporated are: XL Dd at 17.07 per cent (1956), 2.56 per cent and 2.17 per cent (1957); the XL Bb zero per cent (1956), 2.56 per cent (1957); and the XR Dd 19.51 per cent (1956), 2.17 per cent (1957). The control wood showed zero per cent of these inversions consistently. *Heterotic inversions*: the IIR Ee (90.63 per cent in N/S) increased about 10 per cent (24.07 per cent, wood I; 31.62 per cent, wood IV) in 1956 dropping down to control values in 1957 (10.91 per cent, wood I; 11.76 per cent wood IV). The most heterotic inversion III Jj (Pavan *et al.*, 1957) in several places in Brazil did not increase during 1956 but became heterotic in wood I (52.83 per cent in 1957). The hybrid stock has twice as much inversion per individual as compared with control Tainhas and its release raised 1 to 2 per cent mean percentage of inversions in 1956, but in 1957 a reduction in control values occurred.

Most of the genetic novelties introduced in the populations of *Drosophila willistoni* at Tainhas were lost, and the "new" incorporated inversions remained at low frequencies. The genetic homeostasis of Mendelian populations depends on the maintenance of certain levels of heterozygosity (Lerner, 1954) which is attained by a slow process of genotype intergration (Dobzhansky and Wallace, 1953) and coadaptation of genotype systems of balanced polymorphism, all of these processes conditioned by natural selection. The genetic structure of Mendelian population *in naturae* has strong homeostatic properties that tends to reject abrupt or drastic changes even if these are for a further increase in heterozygosity. The levels of heterozygosity show a close connection between adaptive responses and the environment. (This work has been supported in part by grants of the Conselho Nacional de Pesquisas, the Rockefeller Foundation, and the University of Rio Grande do Sul.)

CORLETTE, S. *See* RUDKIN, G. T.

COWDEN, RONALD R. RNA Distribution in Chromosomes during Mitosis and Meiosis. *The Johns Hopkins University, Baltimore, Maryland, U.S.A.*

Cytochemical investigations were undertaken on distribution of RNA in various meiotic and mitotic cells during the division cycle. In all cases prior digestion in pH 6.0 DNAase, 0.01% at 37°C for one hour was used before staining in 0.025% azure B bromide at pH 4.2 for two hours at 37°C. The material used included grasshopper testes, mouse testes and slug ovotestes; for mitoses grasshopper hemolymph cells, onion root tips, and human kidney tissue culture cells were employed. In all cases there was a progressive increase in the binding of metachromatic azure B bromide as the chromosomes condensed. The nucleoli showed a general decrease in size and an irregularity in outline as the cell progressed towards metaphase. The metaphase chromosomes appeared to bind dye maximally with no discernible reduction at anaphase. The telophase chromosomes showed some reduction in basophilia. By the time cleavage was complete, most of the chromosomal basophilia had disappeared, and the nucleoli reappeared.

Although this has been suggested by several past cytological studies, no direct evidence has been recorded in the literature as to direct observation of chromosomal RNA during mitosis and meiosis other than that of Swift *et al.* (1956), who published absorption data and figures of RNA distribution during grasshopper spermatogenesis.

CRAIG, ELLEN MEESON. Point Mutation, Its Rate and Biochemical Effects Considered in Relation to Senescence. (*Private*), *47 Prospect Hill Rd., Camberwell, Australia.*

Theoretically, true point mutation is produced by chemical alteration in the structure of a single gene. It therefore should not require mitosis for its production. The number of point mutations should always be a function of time and the rate of point mutation largely independent of the number of mitoses. This becomes important when considering somatic mutation and its effects on normally non-mitotic essential parenchyma of the well-developed adult organism. As all types of mutation when dominant or homozygous are more often deleterious than otherwise, and as, in terms of biochemical genetics, each confers on its possessor a specific biosynthetic inability, it follows that point and occasionally other somatic mutations summate through the years in each parenchymal cell of a tissue to cause a slow deterioration in its independent synthetic ability. This may be compensated for at first by substances present in sap or circulation, either as diffusible products made in surplus by still unmutated or differently mutated other cells, or as essential substances absorbed. Finally, as somatic mutation continues, all such compensatory mechanisms must break down, and senile deterioration becomes the inevitable outcome, accompanied by a lowering of resistance to disease and an end of the vigorous period of life. Nevertheless, all mutations cannot produce specific biosynthetic inabilities in their possessors. True advance in biochemical complexity could not have been made in evolution unless certain mutations confer added biosynthetic abilities, producing added independence of substances supplied in the environment (Horowitz

Proc. Nat. Acad. Sci. U.S. *31*:153). Increased study of reverse mutation should evolve better reactivating methods whereby somatic cells might be induced to maintain their biosynthetic abilities and vigor in spite of increasing years, thus prolonging the useful part of life.

CRARY, D. D. *See* SAWIN, P. B.

CROSBY, JACK L. The Elucidation of a Problem in *Primula* Evolution by Means of the Electronic Computer. *Botany Department, Durham Colleges in the University of Durham, Durham, England.*

Electronic computers are particularly well adapted to mathematical treatment of evolutionary processes. Micro-evolution is essentially iterative; initial gene frequencies of one generation are altered by a system of forces and the new frequencies are the initial values of the next; the process then repeats itself. Tedious calculations of such processes are greatly simplified by electronic computation, in which iterative calculations are extremely economical of the operator's time; changes in the system of forces can be simulated by automatic modifications of the generating equations, and complicated situations are easily handled.

But nature never follows calculations neatly. Statistical fluctuations may lead to acceleration or complete stoppage, and their consequences when the rate of evolution is changing rapidly are difficult or impossible to estimate. An electronic computer can superimpose random variability on evolutionary calculations; it can therefore run through the same evolutionary process many times but with different results. Such repetition involves no extra work for the operator, and it produces a representation of many isolated populations evolving in the same way, but independently, from similar beginnings.

The evolution of homostyly in *Primula vulgaris*, an account of which appeared in *Evolution* in 1949, will be taken as an example. Certain modifications of the generating equations there given will now be introduced since later work has provided more accurate information about the fertility of the four genotypes involved. The result will be compared with the known population pattern in nature.

The calculating machine has introduced artificiality into evolution studies because the time factor enforces over-simplification. The high speed of the electronic computer allows the consideration of far more factors, and a more realistic and useful approach can be made to natural conditions. This will be valuable to us so long as we prefer the elucidation of evolutionary processes to their use as bases of mathematical fantasy.

CROWE, LESLIE K. The Exchange of Genes between Nuclei of a Dikaryon. *Harvard Biological Laboratories, Cambridge, Mass., U.S.A.*

In intraspecific matings between homokaryotic and dikaryotic mycelia of tetrapolar fungi in the Basidiomycetes, there have been instances in which the homokaryon has been dikaryotized by a nucleus which differed genetically from both nuclei of

the dikaryon. An understanding of this phenomenon has previously been hindered by the lack of adequate marker genes.

Using strains of *Schizophyllum commune* in which markers linked with both the A and B mating type factors are present, dimon matings have been analysed.

Matings in which both nuclei of the dikaryon are compatible with the homokaryon have shown the following reactions: (1) migration of one nucleus of the dikaryon to form a new dikaryon with the resident nucleus in the homokaryotic mycelium (31 cases); (2) migration of the intact dikaryon through the homokaryotic mycelium to supplant the resident monokaryon (6 cases); (3) formation of a new nucleus by exchange of segments, or possibly of entire chromosomes, between the nuclei of the dikaryon (this new nucleus, in which the genotypic characters of the components of the dikaryon are recombined, migrates to form a dikaryon with the resident nucleus of the homokaryotic mycelium) (5 cases); (4) formation of sterile dikaryons in which the genotype of the component haploid nuclei has yet to be determined (5 cases).

It is concluded that new genotypes can arise during the vegetative growth of a dikaryotic mycelium. Since the experiment was designed in a way which gave no apparent selective advantage to recombinant nuclei, they must arise frequently. Recombination may occur either by the fusion of the component nuclei of the dikaryon to form a regular diploid nucleus, or by the overlapping of the spindles during conjugate division resulting in irregular segregation. The latter cause would give rise to many unbalanced nuclei which may account for the sterility of some of the dikaryotic isolates.

CUANY, ROBIN L., ARNOLD H. SPARROW, and ALEXANDRA H. JAHN. Spontaneous and Radiation-Induced Somatic Mutation Rates in *Antirrhinum*, *Petunia*, *Tradescantia*, and *Lilium*. *Biology Department, Brookhaven National Laboratory, Upton, N.Y., U.S.A.*

Plants heterozygous for one or more flower color genes have been treated acutely with X-rays or chronically with gamma radiation from cobalt 60. Small spots or streaks of phenotypically different color appear at random positions on flowers treated as young buds, and occur spontaneously at a low rate. Such changes are referred to as somatic mutations, in a broad sense. The great majority of the mutant areas are quite small but sectors as large as a branch are occasionally produced. These can be propagated asexually into plants bearing only the new mutant flower type. Unfortunately, genetic testing of this material is difficult, since our results so far indicate that the mutant tissue is originally mericlinal, becoming periclinal after propagation, and confined to the epidermis. Such mutations may be of direct value in vegetatively propagated crops, and ways of increasing their frequency and modifying their chimeral nature are being sought.

Antirrhinum, Petunia, Tradescantia, and *Lilium* all show a linear response of mutation rate to gamma dose rate. Average mutation rates expressed per million cells of mature petal, per unit increase in dose rate, are much greater in *Tradescantia* than in *Antirrhinum* or *Petunia. Lilium* shows a still higher somatic mutation rate of the order of 300 spots per roentgen increment of daily dose. This is over two hundred times the rate for *Antirrhinum* or *Petunia* under chronic irradiation. With acute X-ray treatment of *Antirrhinum* flower buds, the average somatic mutation rate per r per cell is 2×10^{-6} above the spontaneous rate of 50×10^{-6}.

While many factors can influence mutation rates for various loci in different plants, the data suggest a possible relationship between radiation-induced somatic mutation rate and chromosome size in the genera tested. Such a relationship would indicate that these so-called somatic mutations are more likely to represent chromosome aberration than point mutation.

(Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.)

Cunha, A. B. da, J. S. de Toledo, C. Pavan, H. L. de Souza, M. L. Pires de Camargo, and L. C. de Mello. A Comparative Analysis of the Effects of Natural and of Radiation-Induced Lethals in Heterozygous Individuals and of Their Frequencies in Natural Populations of *Drosophilia willistoni*. *Universidade de Sào Paulo, Faculdade de Filosofia, Ciencias e Letras, Departamento de Biologia Geral, Sào Paulo, Brazil.*

The hypothesis that the lethal genes present in natural populations, and subjected to natural selection for generations, are less deleterious in the heterozygous condition than newly induced lethals, has so often been repeated that it is now almost accepted as a fact. However, the hypothesis is based almost on theoretical arguments only. We had, in the preparation for our experiments in the islands of Angra dos Reis, an opportunity to test the validity of this hypothesis.

Several lethal genes were induced by X-rays in the second chromosome of *D.willistoni* and 10 of them were taken at random for experiments. At the same time 11 second chromosome lethals were isolated from flies caught in the islands. Flies with the induced and the naturally occurring lethals were raised in the laboratory and released in the islands. No allelism was found with the genes concerned. This test was also used to study the effects of lethals in heterozygous individuals. Individuals heterozygous for two induced lethals and individuals heterozygous for the laboratory marked chromosome 207 (chromosome with a dominant marker gene, a recessive lethal and an induced inversion to prevent crossing-over) and one induced lethal are obtained when the allelism between the induced lethals is tested. In the allelism test between the natural lethals, heterozygotes for two natural lethals and heterozygotes for 207 and one natural lethal are produced. Finally, in the allelism test between natural and induced lethals, heterozygotes for one natural and an irradiated lethal are obtained together with heterozygotes for one 207 chromosome and one natural or one irradiation-induced lethal. These types of crosses permit the comparison of the viabilities for the three types of heterozygotes, heterozygotes for two natural lethals, for two induced lethals, and for two lethals one natural and one induced, taking the hybrid class 207 as a basis. Crosses were made in all possible combinations with the two classes of lethals. Each cross was repeated 5 times with a total of 100 flies analysed for each. The number of the wild-type individuals $\frac{\text{lethal 1}}{\text{lethal 2}}$ for the 500 flies of each cross was taken as the measure of the viability of the heterozygote for the two lethals. From the data obtained it can be concluded that in *D. willistoni,* at least under laboratory conditions, there are no differences in the effect of natural and of X-ray induced lethals on the viabilities of heterozygotes.

The frequencies of the natural and of the induced lethals, used in the experiments above, in natural populations were also studied.

The frequency of the lethal genes in the natural populations studied was found to be of the order of .0005–.0006. The value q for *D. willistoni* populations, taking 2.2×10^{-5} as the mutation rate for this species, is 0.0015.

The experimental value found 0.0005 is much lower than the expected indicating that the lethals are selected against in the heterozygous condition. The hypothesis of genetic drift to explain the low frequency of the lethals is improbable but not totally excluded.

CURTISS, ROY. *See* LEVINE, MYRON.

DALY, KEVIN. An Analysis of Quantitative Variability in *Nicotiana. Department of Plant Breeding, Cornell University, Ithaca, N.Y., U.S.A.*

An investigation of the response of quantitative variability to selection has been initiated with a transgressive line derived from interspecific hybridization. This line originated from the F_1 hybrid of *N. langsdorffii* with *N. sanderae* var. "Sutton's Scarlet" and was advanced by single-plant selection and selfing. The mean of this line exceeded the upper limit of the larger-flowered parent for the selected feature, corolla tube length. To gain information on the nature of gene action controlling the transgressive phenotype, measurement data were collected in a single environment from selfing and backcrossing series with each parent. An analysis of means and variance components has been completed for the selected character and three unselected characters. An indication of the existence and relative magnitude of additive gene action, dominance, and epistasis could be anticipated from this analysis. Expectations for means and variances were developed from a model suggested by Robson (1956) as an alternative to the Hayman-Mather (1955) model for genic interaction under selfing. Three epistatic parameters for digenic interactions, additive $\times$ additive, additive $\times$ dominance, and dominance $\times$ dominance, were estimated in addition to additive and dominance effects. Tests of gene effects showed all estimates to be significantly different from zero. The additive genetic effect exceeded the dominance effect for each of the four characters. The relative magnitude of dominance was found to be less for the selected character than for the unselected ones. Variance component estimates were consistent with results for gene effects in indicating the presence of non-allelic gene interactions. The major portion of the increase in size of the selected line over its larger parent could be attributed to additive gene action and the remaining smaller portion to additive $\times$ additive interaction.

DANSEREAU, PIERRE. Character Variations and Shift of the Ecological Frame in the Species of *Cistus. University of Montreal, Montreal, Canada.*

The prevalence of hybridization in the homoploid genus *Cistus*, and the relative uniformity of the Mediterranean climate throughout the range of the 17 species make them highly responsive to the play of natural selection. Local populations of most of the species have been measured by the mass-collection technique: some, such as *C. bourgeanus* (a southern Iberian endemic) and *C. populifolius*, are

fairly homogeneous; others such as *C. ladaniferus,* are highly variable. The causes of variability may be traced in the first place to the location of many populations very near a present or former bioclimatic boundary (the live oak–semideciduous oak line, for instance). On the other hand, immediate site conditions favour a segregation of types that is strongly accentuated by man's impact on the landscape. Introgression, by and large, has worked towards a more xerophytic cast in the populations of *C. ladaniferus, C. villosus, C. crispus, C. monspeliensis,* and *C. symphytifolius.*

These populations and the related taxa that are also endemics of the Canary Islands show the very same behaviour as the truly Mediterranean species, but this is unfolded within a very different vegetation matrix.

Experimental plants grown in Gran Canaria, at the Botanical Garden in Coimbra, at the University of Michigan Botanical Garden and at the Montreal Botanical Garden, permit an evaluation of the relative role of hereditary characters in these otherwise very plastic plants.

DAREVSKAYA, E. M. *See* BAKHTEYEV, F. KH.

DARK, S. O. S. Some Interactions of *Xanthamonas malvacearum* with the B Series of Black-Arm Resistance Genes in Cotton. *Empire Cotton Growing Corp.* and *Sudan Government, Ministry of Agriculture,, Khartoum North, Sudan.*

In the irrigated Gezira district of the Sudan, the cotton crop is protected from black-arm disease, caused by *Xanthamonas malvacearum,* by very expensive cultural practices, and more recently by the use of 3 genes for black-arm resistance, B_2 (from Upland cotton), B_3 (from *G. punctatum*) and B_6 (from *G. arboreum bengalense*), transferred into the long-staple *G. barbadense* which is grown commercially as two black-arm susceptible varieties called Sakel (S) and Lambert (L).

Degree of resistance of individual plants is graded on a scale 0(immune)-12(fully susceptible), determined by the type and size of leaf spots produced after spraying with a suspension of the bacterium in water under standard conditions.

For many years the reactions of these genes and their combinations were reported to be constant when they had been transferred by repeated back-crossing to the Sudan commercial cottons.

R. L. Knight found that on a *G. barbadense* background, B_2 gave grades 5–7, B_3 gave 4–7, B_2B_3 gave 3 and B_2B_6 gave 4–5. The combination B_2B_3 was considered to give field resistance to the Sudan crop, although water-logging of the plants could break it down temporarily.

In 1956 the resistance of S and L cottons carrying B_2B_3 broke down temporarily owing to water-logging in the Gezira, but in Shambat their mean resistance grades were 5.5 and 5.0 respectively. In 1957 the Gezira had no black-arm outbreak, but in Shambat the mean grade fell to 8.2 and the resistance was indistinguishable from that of B_2 alone.

Evidence from past records and the present season's experiments is conflicting. On the average it seems to indicate that a more virulent strain of bacterium has evolved at Shambat despite lack of selection pressure, for inoculum has always been maintained on a fully susceptible variety of cotton.

DASSAT, PIETRO. Effect of Inbreeding on Milk Production in Sheep. *Istituto di Zootecnica generale, Università Sassari, Torino, Italy.*

The objective of this investigation was to study the effect of inbreeding on milk production in sheep. The analysis was made using the records of a flock of Sardinian sheep (*Ovile Sardo*) set up near Cagliari (Italy) in which a programme of deliberate inbreeding was started before the Second World War to try to produce "superior inbred stock" and to reduce variability. The data available were the first lactation yield in 170 days (standard length) for 564 outbred and 87 inbred animals, and the average fat percentage in first lactation for 381 outbreds and 59 inbreds.

To investigate the effect of inbreeding on yield and fat percentage all the calculations were done within recording years and the differences between outbreds and inbreds, $F = 0.125$, and between outbreds and inbreds, $F = 0.25$, weighted according to the numbers in the groups. The over-all average decline in yield due to inbreeding was 16.54 kg for $F = 0.125$ and 21.03 kg for $F = 0.25$, with a total weight of 7.04 ± 3.7 (approaching significance at the 5% level) and 10.84 ± 5.3 (significant at the 5% level) respectively.

There was some effect on fat percentage and to be exact the decline has a total weight of 0.08 (not significant) for $F = 0.125$ and of 0.32 ± 0.14 (significant at the 5% level) for $F = 0.25$.

The results are in agreement with the existing information about the effect of inbreeding in dairy cattle. About the magnitude of the effect, if we consider an average production of 120 kg milk and 6.5% fat, there is a 9% decline in yield for an F value of 0.25 (i.e., 0.36% change in yield for each percentage increase in the inbreeding coefficient) and a 5% decline in fat percentage.

DAVIES, A. J. S. *See* WYLIE, ANN P.

DAWSON, CHARLES D. R. The Origin of Tingeless May King Lettuce. *A. L. Tozer Ltd., Cobham, Surrey, England.*

May King Lettuce is grown in North European countries in cold frames, glasshouses and as an early outdoor crop. It is early maturing and has good quality, but possesses a defect in that on exposure to cold it develops a reddish-brown tinge on the head. This is disliked on the British market.

A crossing and backcrossing programme has been carried out at Cobham whereby the dominant gene responsible for the reddish-brown tinge has been replaced by its recessive allele introduced from the variety Tozer's Cobham Green. Two new varieties have been produced, Tozer's Mayfair and Tozer's Maybelle, which have all the good qualities of May King, but do not develop any reddish-brown tinge on exposure to low temperatures.

DEGENHARDT, KARL-HEINZ. Analysis of Intra-Uterine Malformations of the Vertebral Column Induced by Oxygen Deficiency in Rabbits. *Institut für Humangenetik der Universitat Münster/Westf., Münster/Westf., Germany.*

In our experimental studies with pregnant doe-rabbits oxygen deficiency proved to be a teratogenic agent adequate for determination of "phase specificity"

("Phasenspezifität") of axial developmental aberrations. Our test series (exposure of pregnant animals to a single test of oxygen deficiency of a few hours duration) indicated the following: the development of axial organ anlagen can be influenced at certain stages, e.g., between the eighth and tenth day of pregnancy (axial gradients were most responsive to alteration on the ninth day of gestation). We found a definite correlation between intensity of the agent (oxygen deficiency), response of the axial (metabolic) gradients and amount of the malformation rate, on the one hand, and between date of the test (during pregnancy) and localization of malformations induced by oxygen deficiency ("cranio-caudales Entwicklungsgefälle"), on the other.

On the basis of these findings, we were able to throw more light on the pathology of the spine, i.e., of its anlage during intra-uterine development. Kladetzky and this author (1955) found that on the ninth day of pregnancy, oxygen deficiency does not primarily affect the vertebral primordium but rather the notochord. Consequently, it is the abnormal cell metabolism of the notochord that induces pathological development of the vertebrae. These results were corroborated by those obtained on Danforth's short-tail mouse, as reported by Theiler (Zurich). There is a similar relationship between pathological function of the notochord and abnormal development of the periaxial mesenchyme (prenatal formation of the disks). Both pathological gene and exogenous agent seem to influence such developmental processes of embryonic organs.

Our observations with rabbits include series of frontal and sagittal sections of embryos submitted to oxygen deficiency (between the ninth and thirty-first day of pregnancy) as well as sectional material of controls.

DeLamater, Edward D. Observations on the Nature of Isolated Bacterial Nuclei. *Section on Cytology & Genetics, Department of Physiology, University of Pennsylvania, Philadelphia, Pa., U.S.A.*

Methods for the isolation of bacterial nuclei have been developed. The methods consist of exposing the cells to bile salts which soften the wall, permitting easier rupture. The released nuclei are subsequently freed from the fragmented cell walls by lysozyme or differential centrifugation. Cytochemical and chemical analyses of the isolated structures indicate the chromosomal nature of the isolated material. Cytochemical evidence indicates the presence of structures comparable to those described in the intact cell, including chromosomes, spindle element, and centriolar-like bodies. Electron microscopy of the isolated material confirms and extends the cytochemical evidence of the nature of the isolated chromosomes and other nuclear structures. The presentation will include photographic evidence substantiating this evidence.

DeMarinis, F. Asymmetry in the Double Bar–Eyeless *Drosophila melanogaster*. *Department of Biology, Fenn College, Cleveland, Ohio, U.S.A.*

Bilateral asymmetry of eye-sizes is studied in the double bar–eyeless females ($BB/+$, ey^2/ey^2) in *Drosophila melanogaster*. The eyeless factor is used to emphasize asymmetry and *BB*-factor is used to reduce the facet-number. A facet count was made on 2,650 flies. The distribution of the eye-size *vs.* number in each group approxi-

mated $y = ab^{-x}$, where y = the number of eyes, x = eye size, and a and b are constants. A further analysis of the classes within each group showed still different type distributions. Each class was determined on the basis of a specific eye size on one side of the head. The tentative results and conclusions derived from these data are: (1) each class gives a different and distinctive type of eye-size distribution; (2) these distributions change with definite regularity from class to class; (3) the same law of distribution operates on either side; (4) developmental events (in relation to facet number) occurring on one side regulate the events occurring on the opposite side; and (5) it has been deduced that the *ey*^{2}-gene acts very early in development. Its action has been estimated as occurring sometime during the early migration of the cleavage nuclei from their central position in the egg to their presumptive eye regions of the oöplasm.

DEMPSTER, EVERETT R., I. MICHAEL LERNER, and NOBRIO INOUYE. Fertility, Viability, and Sex Ratio of the Immediate Progeny from X-irradiated Sperm in Chickens. *Department of Genetics, University of California, Berkeley, Calif., U.S.A.*

In connection with a project for the study of induced genetic variation of continuously variable traits in chickens, experiments were performed to test fertility of eggs from pullets inseminated with sperm irradiated at different dosages, and the viability and sex ratio of the embryos produced. The precise percentages of eggs fertilized were difficult to determine on account of abortive development of unfertilized eggs; different results were obtained when eggs were examined without incubation as compared to results from examination of non-viable eggs after one week's incubation, and the results according to either method of examination were not altogether consistent between experiments. By contrast consistent and regular results were obtained regarding the number of embryos alive at fourteen days divided by the total number of eggs set, when expressed as a proportion of the same quotient used for controls. The relationship between dosage, from zero to 2,000 roentgens, and proportion of surviving embryos closely approximates a simple exponential function. The proportion surviving at 2,000 r units, relative to controls, is 15%. The over-all sex ratio does not deviate significantly from 50%, nor is the variation between experiments significant, as indicated by chi-square tests. The total number of eggs set in these dosage experiments is over 7,000 and the total number of live embryos that were sexed is nearly 4,000.

DEMPSTER, EVERETT R. *See* LERNER, I. MICHAEL, *et al.*

DE SERRES, F. J., and G. KØLMARK. Quantitative Estimates of Radiation-Induced Forward-Mutation Rates at the *ad-3A* and *ad-3B* Loci in *Neurospora*. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

A method has been developed that permits the direct determination of the total number of *ad-3* mutants in a given population of cells after mutagenic treatment. The success of the method depends on the fact that *ad-3* mutants accumulate a

purple pigment in the mycelium, which readily distinguished such mutants from wild type. All conidia surviving mutagenic treatment are grown on minimal medium supplemented with adenine and the purple mutants are recovered on the basis of the characteristic color of the mature colonies. An experiment was performed to study the effect of various X-ray treatments; a comparison was made of the forward-mutation rates obtained with aliquots of a suspension of microconidia (strain K76-OR2-7A) untreated, and irradiated with 4.5, 18, 36 or 72 kr. No purple mutants were recovered in the untreated control in 7.6×10^6 viable conidia, whereas 191 purple mutants were recovered from the four different X-ray treatments. The frequencies of purple mutants per 10^6 survivors at each dose were as follows: 4.5 kr, 2.41 ($16/6.64 \times 10^6$); 18 kr, 5.08 ($26/5.2 \times 10^6$); 36 kr, 33.3 ($76/2.28 \times 10^6$); and 72 kr, 182.5 ($73/0.4 \times 10^6$). The genotypes of individual purple mutants were determined in subsequent heterokaryon experiments using *ad-3A* and *ad-3B* tester strains. Of the 191 purple mutants, 35 were *ad-3A* and 156 *ad-3B* mutants. No double *ad-3A ad-3B* mutants were recovered and no evidence was obtained for the presence of other loci that mutate to phenotypically identical purple mutants either in the *ad-3* region or elsewhere in the chromosome complement. A comparison of the total number of mutants of each type gives an *ad-3A*:*ad-3B* ratio of 1:4.5, indicating a greater sensitivity of the *ad-3B* locus to X-radiation than the *ad-3A* locus. With this strain, maximum forward mutation rates of 9.7 and 2.1 per 10^6 viable conidia treated for the *ad-3B* and *ad-3A* loci, respectively, are expected with a dose of about 55 kr.

DIAMOND, L. K. *See* GERALD, P. S.

DIAMOND, LOUIS K. *See* ALLEN, FRED H., Jr., *et al.*

DICKIE, MARGARET M. Genic Control of the Gonad-Adrenal-Pituitary Triangle: An Hypothesis. *Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.*

Study of postcastrational changes in several inbred strains of mice has shown that, following an injury to the endocrine homeostasis, strains compensate to varying degrees with development of pathological changes in the adrenal glands. Pathological changes are accompanied by biochemical changes, i.e., abnormal adrenals produce sex steroid hormones that stimulate the accessory reproductive organs. Type of compensation is constant for each strain, indicating genic control of the compensatory potential.

To elucidate the genic control of the postcastrational changes, F_1 reciprocal hybrids of some of these inbred strains were examined. Certain strains may compensate identically (phenotypically), but F_1 hybrids of any one of these strains crossed with a different strain reveal some potentials of the parental strains that may have been suppressed in the parental environment but are operative in a new environment. The hybrid potential is not always intermediate between the parental types but may be cumulative so that there appears to be over-compensation (heterosis).

Reciprocal backcrosses of certain hybrids to the parental strains are now being examined. These backcrosses reflect the segregation and recombination of parental and hybrid compensatory potentialities.

It is suggested that the potentialities of the control system, the GAP triangle (gonad-adrenal-pituitary), are of genetic origin and that these compensatory abilities are dependent upon the interactions of at least five polygenic loci. Two of these polygenic complexes are primary, one controlling the general susceptibility or potential of the organism to compensate and the other controlling the normal regulation and interaction of the GAP triangle. In addition there are three specific complexes acting directly and specifically on the adrenal-gonads, the pituitary, and the accessory reproductive organs; and determine, within the limits of the first two complexes present, the quantity and quality of compensation there shall be in the control system, the GAP triangle.

DICKINSON, A. G. Maternal Effects in Cattle. *Animal Breeding Research Organisation, Edinburgh, Scotland.*

In a crossbreeding experiment involving all types of matings among Friesian, Ayrshire and Jersey cattle, the effect of maternal size has been investigated by comparing reciprocal crosses in various ways, using information on weight and body size from birth to 2 years of age. Altogether 13 characters were analysed and a close relationship was found at birth between the relative maturity of the characters and the relative sizes of their maternal effects. Two types of maternal effect are involved depending on whether the crossbred is from the larger or smaller maternal breed, but in both cases the effect diminishes progressively with age of the calf. The main conclusions indicate how an understanding of maternal effects might facilitate a selection programme by permitting selection to be carried out at an early age.

DI PASQUALE, ANNA, and SUSY KOREF SANTIBANEZ. "Pseudotumors" in Natural Populations of *Drosophila melanogaster* and *Drosophila simulans. Istituto di Genetica, Università di Milano, Milan, Italy*, and *University of Chile, Catedra de Biologia, Santiago, Chile.*

The behavior and incidence of "pseudotumors" in descendants of female *Drosophila melanogaster* and *Drosophila simulans* caught in nature were studied for several generations under laboratory conditions. The genetic factors for this trait are being examined in several of the lines of *Drosophila melanogaster*. An analysis for genetic variability is made among these lines.

DISSOSWAY, CAROLYN F.-R. *See* ROBERTS MARGARET R., *et al.*

DLUHY, ROBERT G. *See* FANKHAUSER, G.

DOBZHANSKY, TH. *See* BEARDMORE, J. A., *et al.*

DONOVAN, LORNE STEWART. The Inheritance of Leaf Size in Broadleaf Birdsfoot Trefoil, *Lotus corniculatus* L. *Forage Crops Division, Central Experimental Farm, Canada Department of Agriculture, Ottawa, Canada.*

The inheritance of leaf size was studied in crosses involving the large-leafed "*variety vulgaris*" and the small-leafed "*variety arvensis.*" Results indicate that the genetics of this character is relatively simple in that it appears to be mainly under the control of one gene. This gene shows incomplete dominance and there is evidence that the allelic interaction is geometric in nature rather than arithmetic. Backcross ratios of 1:4:1 and 1:2:1 were obtained which are indicative of auto-tetraploid segregation where the chromosomes pair at random. However, a factorial hypothesis established wholly on this simple basis does not satisfactorily explain the results. Gene interactions, which at present are unknown, are thought to be involved.

Cytological examination of the parent plants showed chromosome pairing to be chiefly as bivalents. Quadrivalent formation was rare. If this species is of autoploid origin as the backcross ratios indicate, the process of reversion to the diploid type of chromosome behaviour is fairly complete and therefore disomic, tetrasomic and di-tetrasomic ratios can be expected.

DOUDNEY, C. O. *See* HAAS, FELIX L.

DOWRICK, G. J. *See* WILLIAMS, WATKIN.

DOYLE, ROBERT J. The Effects of Age of Culture on the Production of Kill and Cytachrom Deficient Yeast by Ultra-Violet Light. *Detroit Institute of Cancer Research, Wayne State University, Detroit, Mich., U.S.A.*

Haploid cultures of an adenine-deficient mutant of *Saccharomyces cerevesiae* were irradiated with ultra-violet light. Improved methods enabled us to show that, through linear portions of survival curves, the production of cytochrome-deficient or "petite" cells varied with age of culture and, in mature cultures, followed a probability relation with dose. The meaning of these curves will be discussed.

DRISCOLL, SHIRLEY G. *See* HSIA, DAVID YI-YUNG.

DURRANT, A. Environmentally Induced Inherited Changes in Flax. *Department of Agricultural Botany, University College of Wales, Aberystwyth, Wales.*

A number of experiments carried out over the past five years have demonstrated that large differences in the weight of flax plants can be produced by the effect of the environment on previous generations of plants. Several environmentally con-

ditioned types, which have remained practically unchanged for at least four generations, have been obtained by growing plants of a single, inbred, self-fertilising variety in various combinations of nutritional and seasonal factors. Although some types are as much as two and three times the weight of others, the plants within the types are highly uniform; for this and other reasons the differences cannot be explained in terms of residual genetic variability within the variety.

The differences are probably due to cytoplasmic change rather than to maternal effects since plant weight is not positively correlated with seed weight. Moreover, conditioned types which were reciprocally grafted remained unchanged in both stock and scion, and crosses of two conditioned types showed some transference of effect through the pollen in both directions. It is likely that the changes are brought about by the effect of mineral supply and temperature/light relations on the relative rates of division of cells and cytoplasmic particles, and the persistence could be due to the altered cytoplasm interacting with the nucleus to establish a new nucleus-cytoplasm equilibrium. The conditioned types do not respond in the same manner to different environments.

Duvick, Donald N. The Regional Localization of Pollen Sterility in Partially Sterile Cytoplasmically Pollen Sterile Maize. *Pioneer Hi-Bred Corn Company, Johnston, Iowa, U.S.A.*

Various degrees of partial pollen sterility occur when certain maize genotypes are transferred into cytoplasms which induce pollen sterility. When individual tassels are examined, one anther at a time over the entire expanse of the tassel, it is seen that in general the anther to anther correlation for percentage of sterile pollen grains tends to increase with increasing proximity of the anthers on the tassel. For example, anthers within the same floret are more likely to have similar percentages of sterile pollen than are anthers in different florets. There is a tendency for "islands" of sterility or conversely for islands of fertility to occur on the tassel. These islands may range in size from two to three anthers to several florets, several spikelets or portions or all of a tassel branch or primary axis. This tendency for regional similarity in degree of pollen sterility will be discussed with reference to the theory that partial pollen sterility is caused by random segregation of particulate entities and also with reference to the effects of external and internal environmental factors on the expression of cytoplasmic pollen sterility.

Ebersold, W. T., and R. P. Levine. Chiasma and Chromatid Interference in *Chlamydomonas reinhardi*. *Biological Laboratories, Harvard University, Cambridge, Mass., U.S.A.*

Four markers, *arginine-1*, *arginine-2*, *p-aminobenzoic acid-2*, and *thiamin-3*, are located in linkage group 1 of *Chlamydomonas reinhardi*. From two crosses involving these markers 1,530 unordered tetrads were analysed with respect to the following considerations: (1) gene order and map distance—the sequence of markers is *arg-1—arg-2—pab-2—thi-3* and the intervals *arg-1—arg-2* (region I), *arg-2—pab-2* (region II), *pab-2—thi-3* (region III) are 5, 15, and 31 map units in length, respectively; (2) position of centromere—the centromere is not within the interval studied, but is approximately 15 units to the left of *arg-1*; (3) chiasma

interference—coincidence values for regions I-II, II-III, I-III are 0.19, 0.31, and 0.70, respectively and thus chiasma interference decreases as the length of the interval under consideration increases; (4) chromatid interference. Among 701 tetrads from cross 1, the ratios of 2-:3-:4-strand double exchanges are: regions II-III, 10:20:6; regions I-III, 8:16:6. These do not deviate significantly from 1:2:1 ($P>0.5$). However, using a method for estimating chromatid intereference developed by Whitehouse certain triple exchanges are used as a basis for "correcting" the frequencies of 2-, 3-, and 4-strand relationships. Negative chromatid interference is indicated since the ratios above are converted to 19.62:14.33:2.05 (regions II-III; $P<0.001$) and to 15.84:13.65:0.51 (regions I-III; $P<0.001$). Regions I-II were not considered since only two double exchanges involving these regions were found. The data obtained from the analysis of 829 tetrads from cross 2 provide no evidence for chromatid interference. The method of estimating chromatid interference used above was not applicable to these data since no triple exchanges were found.

(Supported by research grants from the National Science Foundation (G-2812) and the National Institute of Health (E-1421).)

EGELHAAF, ALBRECHT. *See* KÜHN, ALFRED.

EGGERS, G. W. N. *See* BLUMEL, JOHANNA, *et al.*

EHLING, U., E. KROKOWSKI, and H. G. MEHL. The Uptake of Radioactive Elements in Bones by Skeleton Mutants of Rabbits. *Max-Planck-Institut für vergleichende Erbbiologie und Erbpathologie, Berlin-Dahlem, Strahleninstitut der Freien Universität Berlin, Berlin-Dahlem, Germany.*

The uptake of ^{45}Ca, ^{32}P and ^{89}Sr in the skeleton was examined in Dachs, Pelger and hypoplasia-pelvis rabbits. The genes under investigation were found to cause different kinds of morphological changes. In Dachs rabbits the Dachs gene (*Da*) is responsible for a general reduction of the body size in the homozygote and to a lesser degree in the heterozygote and for significant localized effects upon the endochondral bones of the skull and the limb girdles. In Pelger rabbits the Pelger gene (*Pg*) prevents a normal segmentation of the leucocytes. The nuclei remain in an early segmented stage. In homozygous condition the Pelger gene has a lethal effect. In the super-Pelger (*Pg/Pg*) the nuclear segmentation of the leucocytes is completely suppressed, so that the nuclei are round. The extremities of the super-Pelger rabbits are deformed, ribs and long bones are shortened. In hypoplasia-pelvis rabbits the recessive gene (*hyp*) is responsible for a hypoplasia of the femur and a reduction of the ischium. The reduced ischium impairs development of the acetabulum.

Intensity of the skeletal metabolism depends upon the age of the animals. In comparison with normal rabbits there is an accelerated uptake of phosphorus by the bones of Dachs and super-Pelger rabbits, whereas in hypoplasia-pelvis animals the uptake is delayed. This proved to be true when ^{32}P had been directly injected, as well as in the progeny of female rabbits to which ^{32}P had been applied intravenously. The distribution of ^{45}Ca, ^{32}P and ^{89}Sr within the various bones has been checked by means of autoradiographs.

EIGSTI, O. J. Chromosomes and Speciation as Represented in *Polygonatum*. *Chicago Teachers College, Chicago, Ill., U.S.A.*

The pollen tubes of a triploid show considerable variation in the number of chromosomes from one pollen tube to another when comparing the types among a population obtained from a single flower. This variation is a notable contrast to the constancy exhibited with related species that are diploid and tetraploid, respectively. There are differences between triploids according to the origin of the triploid. This demonstration includes data from cultures obtained over a period of several years. Selected specimens of pollen tubes from diploids, triploids and tetraploids of the genus *Polygonatum* will be available for observation with the microscope. An evolutionary significance of the chromosomal variations as well as the constancy of numbers is noted in these studies.

ELLIS, JACK R. Intergeneric Hybridisation between *Fragaria* and *Potentilla*. *University of Manchester, Manchester, England.*

In a recent series of intergeneric pollinations between the cultivated octoploid strawberry and six British species of *Potentilla*, some fairly vigorous intergeneric hybrids have been raised from crosses where the *Fragaria* cultigen was used as the female parent. The Potentilla species used were *P. fruticosa* ($2n = 14$), *P. erecta* ($2n = 28$), *P. reptans* ($2n = 28$), *P. sterilis* ($2n = 28$), *P. palustris* ($2n = 42$) and *P. anglica* ($2n = 56$), these forming a polyploid series from diploid through tetraploid and hexaploid to octoploid. Four of these *Potentilla* species *P. fruticosa*, *P. erecta*, *P. palustris* and *P. anglica* were highly successful as pollinators, giving large fruits, each with a large number of well-developed achenes. *P. reptans* and *P. sterilis* were completely unsuccessful as male parents, there being no observable stimulation following any pollinations. Germinations occurred in all the hybrid seed though the percentage of seed which germinated and the longevity of the hybrid seedlings varied considerably between the different hybrid cultures.

The most successful of the *Potentilla* species used was $\times$ *P. palustris* because the hybrid seed had the highest germination and the lowest degree of seedling lethality. Many vigorous mature heptaploid hybrid plants ($2n = 49$) were raised from this cross. The cross $\times$ *P. fruticosa* also gave mature hybrid plants having the expected pentaploid number of chromosomes ($2n = 35$). The variety of this species used (grandiflora) was less successful than *P. palustris*, for the hybrid seed had much lower germination and a higher incidence of seedling lethality. In $\times$ *P. erecta*, $\times$ *P. anglica* all the hybrid seedlings originating from these two species of *Pontentilla* died within a few weeks of germination.

All the intergeneric hybrids with *P. fruticosa* were completely sterile, and thus gene exchange between octoploid strawberries and *P. fruticosa* does not seem feasible on account of the prevailing sterility which persisted even in the colchicine doubled hybrid ($2n = 70$). As no fertility details are available for the hybrids with *P. palustris* at the time of writing, no decision can be made on the possibility of using this species in strawberry breeding.

EMERSON, MARY R. *See* EMERSON, STERLING.

EMERSON, STERLING, and MARY R. EMERSON. Protoplasts of *Neurospora crassa*. *Division of Biology, California Institute of Technology, Pasadena, Calif., U.S.A.*

When cultured in media containing appropriate enzymes, for example those present in commercial hemicellulase preparations, the "osmotic" mutant strain of *Neurospora crassa* produces "protoplasts." These protoplasts have something resembling a thin cell wall, but they burst when the osmotic concentration of the medium is sufficiently lowered, leaving a ruptured ghost membrane.

Protoplasts arise as extrusions through pores in the walls of hyphal cells, or directly upon germination of conidia. They are, or soon become, coenocytic, possessing as many as a hundred or more nuclei. In media containing enzymes, protoplasts increase in size and reproduce by rather equal or unequal division, sometimes by a process superficially resembling budding. In the absence of enzymes, and in suitable media, protoplasts sooner or later produce a hyphal-type growth which is resistant to osmotic shock.

Protoplasts of *Neurospora* appear to be especially well suited to certain types of genetic and biochemical studies.

EMSWELLER, S. L., and JOSEPH UHRING. Apogamic Seedlings of Tetraploid *Lilium longiflorum* Thunb. *Plant Industry Station, U.S. Department of Agriculture, Beltsville, Maryland, U.S.A.*

The diploid chromosome number of *Lilium longiflorum* Thunb. is 24. Attempts to obtain triploids by using colchicine-induced tetraploids as maternal parents in crosses with diploids have been made during the past 10 years. In 1954 one capsule that contained 12 seed was obtained. Of these 9 germinated and produced weak seedlings that grew very slowly as compared with diploid or tetraploid seedlings. An occasional diploid seedling flowers in 10 months and a tetraploid in 18 months. Some of the 9 seedlings flowered in 3 years and the remainder during the fourth year.

A cytological examination of these 9 seedlings showed that 8 had 24 chromosomes and the ninth 25. The chromosomes of 6 of the plants were morphologically typical for the species. Plant 7 had a modified B and a modified C chromosome resulting from loss of a piece of the C chromosome including its secondary constriction and attachment of the fragment to the short arm of a B chromosome. Plant 8 also had a modified B and a modified C chromosome, practically identical with those in plant 7, except for attachment of a little larger piece of the C chromosome to the short arm of the B chromosome. Plant 9 had an extra chromosome identified as a modified C indistinguishable from the modified C of plant 7.

The findings indicate that plants 7, 8, and 9 originated from the unfertilized egg cells of the tetraploid. Cytological examination of the tetraploid showed a high frequency of chromosome pairing including bivalents, trivalents, quadrivalents and a few difficult-to-analyse associations of more than 4 chromosomes. Finding of 3 modified C and 2 modified B chromosomes indicates a probable homology between B and C chromosomes. The remaining 6 plants with normal-appearing chromosomes may be of either matroclinal or patroclinal origin. They are actually induced haploids of the tetraploids.

ENDRIZZI, JOHN E. Cytogenetic Study of Frego Bract and a Translocation in Cotton. *Texas Agricultural Experiment Station, College Station, Texas, U.S.A.*

American cultivated cotton, *Gossypium hirsutum*, is an amphidiploid with A and D subgenomes. Heterozygous reciprocal translocations in the amphidiploid commonly show adjacent type of disjunction at metaphase I, and progenies are recovered that are duplicate and deficient for the interchanged segments. Because of the deficiency, these are useful for locating genes on specific chromosomes.

One duplication-deficiency type (Z692) was used as female and crossed to a multiple recessive marker line which included the recessive gene for abnormal bracts called frego. Approximately half of the F_1's had the frego phenotype. Thirteen non-frego or normal F_1's were analysed cytologically and all had normal bivalent pairing at metaphase I as expected, hence, heterozygous for frego. Sixteen frego F_1's were analysed and they had the Z692 arrangement as expected, hence, hemizygous for the frego gene.

Selfs of the normal F_1's segregated as expected in the ratio of 3 normal to 1 frego. Selfs of the hemizygous frego class segregated in the ratio of approximately 3 frego to 1 normal. The latter or normal class was assumed to be due to the transmission of duplicate-deficient gametes through the male and fusing with like gametes in the female. Such individuals would be homozygous deficient for the length of the interchanged segment carrying the frego locus and autotetraploid for the length of the other interchanged segment. This was supported by the fact that a new type of arrangement of four chromosomes was found at metaphase I in this class. Backcross of this group to frego should verify the assumption.

Cytological analysis of hybrids of Z692 arrangement with a D genome species and an interchange involving only A genome chromosomes showed that the interchange is between chromosomes in the A and D subgenomes and that the frego locus is in an A chromosome.

The double duplicate and deficient type is fully fertile and shows that interchanges may be useful for adding and/or subtracting segments in the chromosome complement of cotton with the intent of improving its economic characters.

The fact that plants homozygous deficient for the frego locus have normal bracts is some indication of the nature of the "duplicate homologue" in the amphidiploid.

ENGEL, KARL-HERMANN. Effect of Inbreeding and Crossbreeding in *Solanum tuberosum*. *Deutsche Akademie der Landwirtschaftswissenschaften zu Berlin, Institut für Pflanzenzüchtung, Rostock, Germany.*

Comparing the investigations of parents and their in- and cross-breedings for some typical characters, such as yield, starch content, flesh colour and tuber shape, we try to explain the results by Mendelian segregations on the hypothesis of both dominance and superdominance. We are still working on this problem.

ESSER, K. The Significance of Semi-Incompatibility in the Evolution of Geographic Races in *Podospora anserina*. *Botanisches Institut der Universität Köln, Köln-Lindenthal, Germany.*

In Fungi and flowering plants, where the phenomenon of incompatibility is determined by various genetic and physiological mechanisms, genetically identical

gametes are invariably incompatible. Heterogenetic individuals, except for rare cases, are compatible. Up to the present time the belief was justified that incompatibility serves as a mechanism for the prevention of inbreeding.

In contrast the incompatibility in inter-race crosses of *Podospora anserina* differs in its genetic basis, its realization and its evolutionary effect. This incompatibility is based on the occurrence of semi-incompatibility (SI) between genetically heterogeneous sex organs. Thus inbreeding and isolation of races are promoted.

This isolation cames about in two separate ways, both of which depend on the same action of the semi-incompatibility genes. (1) In crosses involving SI the number of hybrid perithecia is reduced to half because only one of the two reciprocal crosses yields fruiting bodies. The cumulative action of the two SI mechanisms can completely block sexual reproduction when both reciprocal crosses are blocked. (2) If hybrid fruiting bodies arise in SI crosses, genetic equilibrium among the progeny is significantly altered in favour of certain genes. The result is that in the homocaryon specific mutations arise, whereas in the heterocaryon particular nuclei are eliminated. For the latter, the existence of binucleate spores which contain two of the four meiotic products is a prerequisite.

EVANS, ALICE M. Grafting and Hybridization in the Genus *Trifolium*. *Welsh Plant Breeding Station, Aberystwyth, Wales.*

Previous workers have shown that *Trifolium* is a genus displaying very little interspecific compatibility. Hence a new approach was essential and new techniques had to be evolved in any worthwhile attempt to overcome the barriers to crossing within the genus. Michurinist geneticists in the U.S.S.R. have claimed that "vegetative rapprochement" can be used for making distant crosses compatible. In consequence such grafting methods were applied in the genus *Trifolium*.

Reciprocal grafts were made between selected species at different levels of ploidy, and it was found that some scions developed normally, others were stunted, while others died very soon after grafting. Where the grafts succeeeded, hybridization between stock and scion was attempted and the success of these hybridizations was compared with that obtained by crossing ungrafted plants. It became evident that grafting prior to crossing did not increase crossability of species, but where stock $\times$ scion and scion $\times$ stock crosses were successful, it was also possible for the control plants to be successfully hybridized.

The data also showed that the grafting technique served as a sieve in that those genotypes that did not graft likewise did not hybridize. Thus on retaining for hybridization only those genotypes which were compatible as grafts, there was a selection for the most compatible genotypes. Graft compatibility hence became an indication of the relationship between species. The significance of these results will be discussed.

EVANS, E. BURKE. *See* BLUMEL, JOHANNA, *et al.*

EVANS, LAURIE E. *See* JENKINS, B. CHARLES.

Fahmy, O. G., and Myrtle J. Fahmy. Differential Cell-Stage Response to Mutagens. *Chester Beatty Research Institute, Royal Cancer Hospital, Pollards Wood Research Station, Chalfont St. Giles, England.*

The effect of the cell stage during spermatogenesis in *Drosophila melanogaster* on the mutation yield (as regards sex-linked recessive lethals) under the effect of different mutagens has been investigated by the "brood" technique. Males were irradiated or injected with a single dose of a chemical mutagen, and their progeny was fractionated by repeated matings to a succession of virgin females; the mutation rate in each fraction (or brood) was determined separately. The fluctuations in the brood-mutation curve were analysed with a view to the elucidation of how far they were a function of the cell stage response during spermatogenesis, and whether this response was a function of the mutagen.

Variation in the brood-mutation curve sometimes occurred with the same mutagen owing to factors inherent in the technique itself, mainly as a result of: (a) cell killing at high doses of some chemical agents, (b) variation in the speed of germ line differentiation, and (c) alteration in the experimental procedure particularly the brood period. In spite of these spurious variations, however, it was possible to detect some gross trends in the brood-mutation curves which seemed to be characteristic of the various agents. X-radiation, tri(ethyleneimino) triazine, diepoxybutane, as well as the acid nitrogen-mustards (with carboxylic side chains), were shown to produce their maximal mutagenic effect on the earlier spermatids. The basic nitrogen-mustards (i.e., amino-acid derivatives), on the other hand, produced high mutagenic effect on all stages of spermatogenesis, with slightly higher selectivity on the spermatocytic and late spermatogonial stages. The methanesulphonic esters were highly active on the sperm and late spermatids and their effect gradually decreased in the earlier germ cell-stages. Two compounds, 2-chloroethyl methanesulphonate and S-chloroethyl cysteine, acted selectively on the spermatogonial stages.

It was, therefore, concluded that mutagenic response depended on the physicochemical state of the genetic material during germ-cell differentiation, and that the spectrum of this response varied with the mutagen.

Fahmy, Mytle J. *See* O. G. Fahmy.

Failla, G. Relation between Spontaneous Mutation Rate and Mortality Rate. *Radiological Research Laboratory, Columbia University, New York, N.Y., U.S.A.*

When the logarithm of the age specific death rate, r, is plotted against age, t, in years, the portion of the curve beyond middle age is approximately a straight line, represented by the equation $r = r_0 e^{-\alpha t}$, where α is the slope. If it is assumed that aging is due to somatic mutations, it may be shown that α represents the average spontaneous mutation rate per cell per year for the cells concerned. A representative value of α for human populations is 0.085. If the diploid cell contains 4×10^4 genes and one generation is 30 years, the mutation rate derived from this value of α is 6.4×10^{-5} per gene per generation. The corresponding rates for mouse, rat, and *Drosophila* at 25°C, derived from published mortality data, are all about 7.8×10^{-5}. Obviously the close agreement is due in part to the choice of the number of genes per diploid cell (mouse and rat, 2×10^4; *Drosophila*, 10^4) and the generation time (mouse, 200; rat, 250; *Drosophila*, 12 days).

The product of a and the lifespan, which represents the average number of mutations that have occurred per cell at the end of the lifespan, is nearly constant for the four species (8 to 6) and does not involve any juggling of figures. Thus the spontaneous mutation rate per cell and per unit time is (roughly) inversely proportional to the lifespan. If the increase in mortality rate with advancing age is due primarily to the accumulation of somatic mutations, the lifespan is set by the inherent stability of the genetic system of the species, as represented by the spontaneous mutation rate in somatic cells.

FALCÃO, D. N. *See* CAVALCANTI, A. G. LAGDEN, *et al.*

FALCONER, D. S. Genetic Correlation between Growth on High and Low Planes of Nutrition: An Experiment with Mice. *Institute of Animal Genetics, Edinburgh, Scotland.*

The theory of genetic correlation may be applied to the problem of interaction between genotype and environment, and leads in principle to a solution of the problem of how much of the improvement gained by selection carried out in one environment will be maintained if the improved strain is transferred to another environment. Any phenotypic measurement made in two different environments may be regarded as measuring two different "characters," and the degree of genetic similarity between the two characters, arising from pleiotropy, may be expressed as the genetic correlation. Knowledge of the genetic correlation and of the heritabilities of the two characters should then allow one to predict the improvement to be expected in character A if selection is made for character B. An experiment with mice was carried out with the object of testing this idea. The measurement made was of growth between three and six weeks, and the two environments in which growth was measured were high and low planes of nutrition. Two lines were selected for increased growth, one reared on high plane and one on low. Similarly two lines were selected for decreased growth. Selections were made from first litters, and second litters were reared in the other environment. Thus the response of both characters was measured in every generation. The genetic correlation was measured from a comparison of the direct response with the correlated response. Four such comparisons could be made: from the two characters, and from the two directions of selection. If the theory were adequate to deal with the problem, these four estimates would be consistent. The estimates from the two characters agreed well when based on the divergence between upward and downward selection. The genetic correlations were then 0.63 and 0.50. But the four estimates derived from consideration of the upward and downward responses separately did not agree at all. The discrepancy was due to asymmetry of the responses in the two directions, whose cause has not yet been traced, but is likely to be connected with maternal effects.

FALEK, ARTHUR. Hand Preference in Families. *New York State Psychiatric Institute, Department of Medical Genetics, New York, N.Y., U.S.A.*

A study of hand preference has been conducted in families of New York City school children (1) to determine the cause and total expressivity range of hand preference, (2) to explore the apparent interaction of genetic and conditioning factors, and (3) to establish, if possible, a family pattern of hand dominance which

would explain the distribution of right- and left-handedness in children of the four mating combinations.

Of 5,118 families responding to a preliminary take-home questionnaire, a sample of all left to left matings and an equal number from each of the other three mating types were chosen. The given families were further examined by means of a home demonstration test, motor tests, and extensive interviews.

Statistically significant hand differences were observed in the children of the four parental matings by the demonstration test, and if the ambidextrous population was removed, by the motor tests as well. Only in the matings of right-handed fathers and left-handed mothers was the proportion of left-handed children significantly and consistently larger than the observed rates in the other three types of matings. Re-evaluation of the home demonstration test according to the Rife technique, while confirming his observations of an increase in the number of left-handed children in those families where one or both parents are left-handed, was nevertheless in agreement with the other findings of this study.

Evidence is presented that the hand preference of an individual is a result of both his genetic endowment and his early training in the home.

FANKHAUSER, G., and ROBERT G. DLUHY. Relative Constancy of Somatic Chromosome Numbers in Larvae of the Salamanders *Triturus viridescens, Pleurodeles waltlii*, and *Eurycea bislineata*. *Department of Biology, Princeton University, Princeton, N.J., U.S.A.*

Aceto-orcein squashes were made of the liver of larvae in different stages of development. A 15-minute pretreatment in 38% Niu-Twitty solution was found to facilitate the spreading of the chromosomes. A single liver may yield as many as 100 mitotic figures of which about one-third are countable.

In the liver of young *Triturus viridescens* larvae ($2n = 22$) at least 80% of the mitoses were found to be diploid, about 15% tetraploid. The remaining metaphases contained either 21 or 23 chromosomes, or were so thoroughly scattered by the squashing that no reliable count could be made.

In *Pleurodeles waltlii* ($2n = 24$) the liver of young larvae showed approximately the same percentages of diploid and tetraploid metaphases as in *Triturus*. Occasional mitoses with either 23 or 25, and a single figure with 26 chromosomes were found. No other deviations in chromosome number were encountered. Counts in a smaller number of *Eurycea bislineata* larvae ($2n = 28$) also revealed a great preponderance of diploid metaphases, and a small number of tetraploid figures.

The diploid chromosome complement of the *Pleurodeles* includes a pair of satellite chromosomes. Their relation to the nucleoli has not been investigated so far. However, studies of tailtip preparations stained *in toto* with Harris's hemalum have shown that each haploid chromosome set must contain two nucleolar organizers, since the maximum number of nucleoli present in epidermis nuclei of the tailfin is four for diploid, six for triploid, eight for tetraploid, and ten for pentaploid larvae. This is in contrast to the condition found in the axolotl, where the corresponding numbers of nucleoli at different levels of ploidy are two, three, four, and five (Fankhauser and Humphrey, 1943).

FARTHING, B. R. *See* LEGATES, J. E., *et al.*

Fechheimer, N. S. and L. O. Gilmore. Factors Affecting the Deoxyribose Nucleic Acid Content of Bovine Germinal Nuclei. *Department of Dairy Science, Ohio State University, Columbus, Ohio, U.S.A.*

Cytospectrophotometric measurements of Feulgen-stained DNA and chromosome counts were made on testicular samples taken from 18 bulls representing 5 dairy breeds of cattle. At the time of sampling the bulls ranged from 12 to 15 months of age. One testis of each bull had been injected with colchicine at 3 to 5 months of age; the other testis was used as a control. Feulgen-DNA determinations of 20 spermatogonia, 20 primary spermatocytes, and 20 spermatids were made from stained preparations of each testis. The DNA values obtained from each slide were corrected for variation in staining intensity by use of a section of *standard* liver on each slide.

Statistical analyses of these data show that there was no difference between colchicine-treated and untreated glands or between samples from bulls grouped by age. There were significant differences in mean DNA content, cell to cell variability of DNA content among breeds, and among individual bulls belonging to the same breed. A statistically significant discrepancy from the theoretical 1:2:4 ratio of the mean DNA content of the nuclei of spermatids, spermatogonia, and primary spermatocytes was found. These differences in nuclei of the germ line cells can be explained by an inconstancy of chromosomes within or among animals, or by the presence of two types of DNA in cattle nuclei with one of these types not associated with genetic determination.

Fejer, S. O. A Gene for Femaleness in Hermaphrodite *Lolium perenne* L. *Grasslands Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand.*

An inbreeding programme in perennial ryegrass, coupled with selection, resulted in a high-tillering "pasture" and a low-tillering "hay" line. Selection in the first two generations was for herbage production, for quantitative inheritance studies. It had to be switched to high self-fertility in the following generations, but this left the main characters unchanged. The gene reported here emerged in two plants out of twenty-two in the third generation of the "pasture" line and had not been observed earlier. All flowers examined in these plants appeared to be purely female and the three anthers were replaced by three ovary-like structures. From open-pollination of these two plants a few seeds were set, not more than one per floret, and all the plants from these were normal and vigorous. Only two of these plants were self-fertile enough for further studies and twenty plants of each selfed progenies were grown to maturity. Both of these progenies contained plants with all flowers totally female, 5 and 6 out of 20, a nearly perfect 3:1 segregation ratio. All the 11 abnormal plants set again some seeds from open-pollination, but crosses of these plants to related normal plants from both lines were fully sterile, while normal plants in one progeny were nearly fully self-fertile. It is suggested that a factor, changing sex expression, is segregating here like a single recessive gene and the homozygous recessive "female" plants are sterile if pollinated by a plant carrying the gene in heterozygous condition. Another recessive gene segregating in one of the lines caused lethal albinism, but seemed to be independent.

Genes affecting sex expression in Gramineae were reported by Jones in

dioecious maize, and Frankel and Fraser found a "base sterile" gene in hermaphrodite wheat which, according to Barnard, forms a series of changes towards "femaleness." However, a complete change due to one gene or gene complex is not known and the present case may support the hypothesis of Baker on simple sex control by auxins. It may also have complications for the developmental anatomy of floral parts and the operation of self-sterility systems.

FLORY, WALTER S., Jr. Speciation, Mitotic Chromosome Number, and Karyotype Evolution in the Amaryllidaceae. *Blandy Experimental Farm, University of Virginia, Boyce, Va., U.S.A.*

Cytological information is available for some species of almost half the known genera of the Amaryllidaceae. A predominating basic number 11 occurs in about half the genera known cytologically, and is the only known basic number of all or most species in 12 genera. Both static and dynamic genera occur. *Amaryllis* and *Crinum* represent static genera; in each the species are clearly differentiated and the cytological picture essentially constant.

This report deals chiefly with dynamic groups of which *Hymenocallis* and the genera of Zephyrantheae are illustrations. Taxa differentiation is here often difficult and chromosome disparities frequent. Evolution in these active genera results—singly or perhaps sometimes in combination—from hybridization, polyploidy, or chromosome changes with resulting aneuploidy. Genic changes presumably play some part. Ready production of viable seed as well as several types of asexual propagation tends to promote survival of newly evolved variants.

Artificially produced hybrids in Zephyrantheae (*Zephyranthes* × *Cooperia* species; *Z.* species hybrids and backcrosses; *Habranthus brachyandrous* × *H. robustus*, etc.), often between parents with differing chromosome numbers, illustrate experimentally a putatively rather frequent method of originating new natural types and taxa. Artificial, and putative natural, hybrids occur in *Hymenocallis* and other genera.

In the closely related genera *Zephyranthes, Cooperia* and *Habranthus* a euploid series of somatic chromosome numbers runs from 12, through 18, 24, and 48 to 54 and probably 72. Other natural taxa occur with numbers of 14, 25, 28, 38, 46 and 47 plus a fragment. Artificially produced derivatives have other numbers.

Somatic numbers from 38 through 40, 42, 44, 46, 48, 50, 52 and 54 to 69, 70 about 84 and 88 to 98 occur in various taxa of *Hymenocallis*. In hybrids or in *Ismene* section, additional taxa with numbers of 45, 56, 58 to 60, 74 to 80, and 104 to 110 are known. Taxa with 46 (or 69) or 40 chromosomes, all metacentric, are most prevalent. Telocentric chromosomes occur in complements of most taxa with other numbers. These other numbers usually reduce to 46 (or 69)—or 40—if half of the number of telocentrics is added to the number of metacentrics.

FOCKE, RICHARD. A New Way of Explaining the Frequency of Polyploid Plants in Certain Habitats. *Institut für Landwirtschaftl. Biologie, Abt. Genetik der Universität, Rostock/Mecklenburg, Germany.*

From the initial consideration that the "period of persistence" of mutated genes partly depends upon the degree of polyploidy and the mutation rate, some con-

clusions are drawn regarding the formation of favourable combinations of genes and the further evolution of forms caused by them. Observations and calculations are added to support the conjecture that there is a relation between the geographic distribution of the polyploids and the natural ionizing radiation occurring in those regions.

FORD, CHARLES E. *See* HAMERTON, JOHN S.

FORD, D. K. *See* YERGANIAN, G., *et al.*

FORD, LEE. Possible Pleiotrophic Effects of the "Gray" Gene in Collie Dogs. *Pacific Lutheran College, Parkland, Washington, U.S.A.*

Studies currently being done on the "gray" coat color gene, *dd*, in collie dogs, would indicate either that the gene in homozygous recessive condition has pleiotrophic effects, or that there is a very close linkage involved. To date in examination of 40 pedigrees involving 84 "grays," there have been found no examples of what could be termed "crossovers," hence at present pleiotropism is suspected. Effects of the *dd* condition include extreme dilution of pigment, resulting in "gray" coat color, decreased mentality to "moron" level, drastic effect on erythrocytes, leucocytes and platelets, possible vitamin-synthesis deficiency, failure to develop any kind of immunity. One difficulty in the study has been separation of the "gray" genes, *dd*, which result in the abnormal pups, which generally die either at birth or at a few months of age, with the "dominant gray" coat color caused by the dominant gene, *G*, which produces perfectly normal pups. The homozygous recessive *dd* pups result from two normal parents. The "dominant gray" pups must have one gray parent. Variations in effect of genes on actual phenotype further complicate the problem.

FORSTHOEFEL, PAULINUS F. The Development of Tail Anomalies Found in Mice Homozygous for the *Luxoid* Gene. *University of Detroit, Detroit, Mich., U.S.A.*

The *luxoid* gene, besides having other effects on the skeleton of the mouse, causes reduction of the centra of one or more vertebrae in the tail of homozygotes. The affected vertebrae tend to slip out of line with the remaining normal vertebrae, resulting in externally observable kinks. Sometimes adjacent vertebrae are partially fused and rarely the portion of the tail beyond a modified vertebra is missing. Study of the embryological development of the tail in *luxoid* homozygotes traced the reduction of the centra of the vertebrae to reduced somites formed during the budding of the tail. The partial fusion of adjacent vertebrae was traced to the eccentric formation of nuclei pulposi from the notochord. The eccentric formation may in turn be caused by incomplete formation of the sclerotomic fissure. Loss of the end of the tail was traced to the common formation of large blebs in the vascular system which sometimes lead to occlusion of the circulation of the tail and necrosis of the tail distal to the point of occlusion. Other anomalies found in the development of the tail were partial doubling involving notochord and somites

and duplications of the nerve tube. Occasionally the duplications of the nerve tube affected higher levels of the nerve cord, including the cervical region and brain. A case of pseudencephaly observed in one *luxoid* homozygote may be related to the duplication effects on the nerve cord. (Investigation supported by a grant from the U.S. National Science Foundation.)

FOSTER, M., and L. THOMSON. The Effects of Substitution at the Leaden (*ln*) Locus on Melanogenic Attributes of the Mouse. *Yale University, New Haven, Conn., U.S.A.*

In our continuing investigation of the genetic control of mammalian melanogenesis, we have recently begun to study the effects of substitutions at the leaden (*ln*) locus in the mouse on natural melanin content, on tyrosinase and dopa oxidase activities, and on ability to darken upon *in vitro* incubation in the presence or absence of exogenous melanogenic substrates.

Although both leaden (*lnln*) and maltese dilution (*dd*) genotypes are very similar in appearance owing to clumping of pigment and reduced melanin content compared with their intense eumelanic counterparts (*Ln* or *D*, respectively), the skins of these two genotypes differ in certain metabolic and physiologic respects, especially notably on a black (*B*) background. (1) Leaden (*lnln*) black skins, while no darker than dilute (*dd*) black skins, are more active than dilute (*dd*) black skins in both enzyme activity and ability to undergo *in vitro* darkening. (2) So far, only in the leaden (*lnln*) background, in contrast to dilute (*dd*) and other backgrounds, have we observed the melanogenic superiority of black (*B*) over brown (*bb*) skins when incubated in the presence of exogenous substrate. (Work supported in part by NSF Grants G-2110 and G-5028 (Foster), by an Institutional Research Grant from the American Cancer Society, and by USPHS Grant C-2838 (Lerner).)

FOSTER, T. S. *See* STERN, HERBERT.

FOX, ALLEN S. Immunogenetic Studies in Relation to Problems of Protein Synthesis. *Michigan State University, East Lansing, Mich., U.S.A.*

On the basis of studies disclosing interaction between non-alleles, alleles, and pseudoalleles in the determination of antigenic specificity in *Drosophila melanogaster*, we have suggested that protein synthesis might be divided into two gene-controlled stages: (1) synthesis of a polypeptide pre-protein, and (2) formation of cross-linkages and folding of the chain.

Changes in X-chromosome dosage result in qualitative shifts in antigenic pattern. Comparison of XY and XO males with XX and XXY females discloses that the former possess two proteins (♂–1 and ♂–2) of distinct antigenic specificity lacking in the latter, which possess instead two proteins (♀–1 and ♀–2) of different specificity. This effect is attributable to the difference in dosage of one or more euchromatic loci on the X, possibly the sex-determining loci themselves. Some sort of interaction mechanism must be involved.

The heterochromatic Y-chromosome has no detectable effect on the antigenic specificity of proteins. However, the physical properties of a particular protein are subject to a maternal influence of the Y. When a female lacks a Y-chromosome, her progeny exhibit an antigenic specificity (Y–1) associated with a protein capable of inducing antibody formation in rabbits, capable of uniting with and precipitating specific antibody, and resistant to lyophilization. When a female possesses a Y-chromosome, her progeny exhibit the same antigenic specificity (Y–1), but it is associated with a protein incapable of precipitating specific antibody and is frequently destroyed by lyophilization, although it is capable of inducing antibody formation and of uniting (inhibiting) with the antibody. The difference would seem to be attributable to grosser features of protein structure than are concerned with antigenic specificity, which must be the product of one or more euchromatic loci.

The X–chromosome dosage effect on specificity may be associated with one of the two postulated stages of protein synthesis, and the maternal Y-chromosome influence may be with the other. Perhaps euchromatin in general is concerned with the control of protein specificity, while heterochromatin is concerned with gross protein structure (size, folding, cross-linkages, etc.), both effects being mediated by RNA. Schultz's observation that the Y-chromosome influences the type of RNA synthesized in the oocyte is probably related to these findings, and serves to connect them with the biochemical evidence that protein synthesis involves activation of amino acids by ATP, condensation of activated amino acids into a polypeptide chain presumably on an RNA template, removal of the polypeptide chain from the RNA surface, and the formation of cross-linkages and foldings.

FRACCARO, MARCO. Breeding Structure of Human Populations: Data from a Swedish Parish. *The State Institute for Human Genetics, Uppsala, Sweden.*

The parish of Ovanåker, in middle east Sweden, is a rural community; some industrial activity has however developed in recent years. Total area is 520 sq.km., 80% of it covered by forest. Communications are good with no geographical barriers. The population was 4,212 in 1900 and 7,239 in 1951. Crude migration rates show that the increase is due more to higher fertility than to immigration. Separate examination of the 1,262 marriages contracted in the period 1850–99 and the 2,098 contracted in the period 1900–44 has been made for all or part of the variables, locality of origin of the mates, geographical distance between them and frequency of consanguineous marriages.

The estimate of the frequency of marriages with at least one partner coming from another parish provides an analysis of migration of more interest than crude migration rates. This has been done for both periods 1850–99 and 1900–44 taking into account the sex of the resident partner. Of the marriages 83% are between partners both born in Ovanåker in 1850–54 but only 41% in 1900–44. No significant difference as to the sex of the resident partner is observed in the period 1850–99, while in the period 1900–44 the resident partner is more frequently the husband. The distribution of the geographical distances between partners is analysed taking into account the sex and age of the partners, and density of population at various distances. A first analysis of the distributions does not provide a simple model for the parameter "geographical distance."

The frequency of first cousin marriages is estimated at 0.57% in the period

1900–44, based on the registration of marriages over three generations. Distribution of a rough estimate of geographical distance between partners of these marriages has been obtained. The genetic implications of these demographic data are discussed.

FRANZKE, CLIFFORD J. *See* ROSS, JAMES G., *et al.*

FRANKEL, O. H., RUTH GANI, and ANNE MUNDAY. Two Independent Gene Systems for Floral Induction in Wheat. *Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia.*

The lowest two (or three) florets in each well-developed spikelet of *Triticum vulgare* are invariably fertile; but in a series of speltoid mutants with progressive basal sterility, the lowest one, two or three flowers are reduced or missing. Fertility is restored by re-introducing the "vulgare inductor": all *T. vulgare* types in crosses mutant $\times$ *T. vulgare* are normal. Flower development in *T. vulgare* is thus activated by a single dominant fertility inductor (I^+) which most likely is part of the *T. vulgare* inductor, Q.

There is, however, a second system for flower induction in *T. vulgare,* revealed in the absence of I^+ (and Q), i.e. in speltoids. Induction of the lowest flower requires 3 genes, of the next a fourth, of the third at least a fifth; these are minimum assumptions. One is dominant for fertility, the rest have no dominance. Of the *T. vulgare* tested, 4 carry at least 1 (1 of them 4) sterility alleles. Presumably there are further genes, and higher sterility grades; speltoids with only an occasional grain in spikelet tips occur.

We thus find 2 gene systems: one monogenic, with dominance, part of a "supergene" of vital significance; the other polygenic, without clear dominance, distributed, cryptic in action. The polygenic system has no obvious effect on fertility in *T. vulgare;* but *T. vulgare* carrying four sterility alleles exhibits a trace effect of flower reduction.

Since the monogenic system performs the function effectively, why does the polygenic system exist? (1) The polygenic system may have pleiotropic effects of which there is no evidence. (2) The monogenic system may be a recent acquisition, the polygenic an older system, now in disuse and removed from effects of selection. If, according to McFadden and Sears, Q (hence probably I^+) was transferred from a free-threshing Lake Dweller wheat to *T. spelta* in historical times, the former brought the monogenic, the latter the polygenic system. Whatever the origin, the evidence suggests that the monogenic system took charge and the polygenic system is in a degenerative state.

FRASER, A. S., and R. B. DUN. Selection for an Invariant Character "Vibrissa Number" in the House Mouse. *Commonwealth Scientific and Industrial Research Organization, Animal Genetics Section, Zoology Department, Sydney University, Sydney, Australia.*

"Vibrissa number" in the house mouse is scored by addition of the number of vibrissae in the five minor groups found on the head and fore-limbs of the mouse—

a total of 19 vibrissae. Inbred lines A_w101 and CBA showed no variation in "vibrissa number" and a parent stock based on these two lines was used in an attempt to break down the invariant vibrissa development.

In order to produce phenotypic variance in "vibrissa number," the sex-linked, semi-dominant gene Tabby (*Ta*) was crossed into the selection stock. The average "vibrissa number" in (*Ta*+) females and (*Ta*) males is 15.1 ± 1.9 and 8.7 ± 1.6. High and low lines were established by selecting within this uncovered variance and in seven generations the selection lines have been separated four standard deviations (Ta+ female score).

The first wild-type mice with low "vibrissa number" appeared in generations 5 and 6 of the low selection line and four of these mice had lost a post-orbital vibrissa—a site which was found to be invariant in an examination involving 6,000 mice. In generation 6, four mice with increased vibrissa number appeared in the high line. Three of these mice had an extra supra-orbital vibrissa and one mouse had four inter-ramal vibrissae.

These results show, first, that there is genetic variation in vibrissa development which is not apparent in unselected mice in the absence of *Ta*. Secondly, it has been possible to breed wild-type mice with both more or less than the standard number of vibrissae by selecting within the variation which becomes apparent when *Ta* is introduced. Although the genetic basis of these changes is unknown, it seems reasonable to suggest that the polygenic system controlling vibrissa development has been altered by selection.

FRASER, F. C. *See* TRASLER, DAPHNE G.

FREESE, ERNST. Specific Mutations of Phage T4 Induced by Isoadenine. *Biological Laboratories, Harvard University, Cambridge, Mass. U.S.A.*

An understanding of mutagenesis on the molecular level requires the correlation of genetically localized mutations with well-defined chemical changes in the hereditary material. Among the large number of mutagens, those chemicals are of special importance which produce a specific and localized chemical change on DNA. Especially promising are those base-analogues which can be built into DNA instead of a normal base. The structural model of DNA, presented by Watson and Crick, suggests that only those base-analogues can be mutagenic, by direct incorporation into DNA, which are able to form at least two hydrogen bonds with either of two normal DNA bases. A given purine analogue, for example, is expected to compete readily with one of the normal purines for incorporation into DNA; however, rarely it may behave like the other normal purine and pair in its place with the complementary pyrimidine. Each of these rare pairings alters the normal sequence of DNA nucleotides and causes a mutation. The mutation frequency is greater than that of spontaneous mutations if these mistakes occur much more frequently than the spontaneous mutations. Such a direct mutagenic action of a base-analogue is possible only if it can be converted by the enzyme system present in the cell into the direct DNA precursor. Applying these considerations as well as using the partially known steric requirements of those enzymes which are responsible for the production of DNA precursors, a small number of base-analogues have been selected and

tested for their mutagenicity. It was found that 2-aminopurine (= isoadenine) exerted a strong mutagenic effect upon phages T4. A large number of independent (*r*) mutants of T4 have been isolated and genetically mapped. The fine structure mapping within the *r* II cistron showed that sites of high mutability exist which are different from the ones found among spontaneous *r* mutants. One of these sites coincides with one of the sites of high mutability among the *r*-type mutants induced by Bromouracil while the other sites are different. The significance of these results will be discussed.

FREIRE-MAIA, A., N. FREIRE-MAIA, and A. QUELCE-SALGADO. Genetic Aspects of Achiropody. *Laboratory of Genetics, Faculty of Philosophy, University of Paraná, Curitiba, Paraná, Brazil.*

Only one instance of familial achiropody has been reported in the genetic literature (Peacock, Eug. News *14*: 46–47; Tucker, Eug. News *15*: 59–60; Cook, Eug. News *15*: 86–87; and Bohomoletz, Eug. News *15*: 143–145. Owing to the extreme rarity and highly mutilating effects of the condition this family has been widely cited. Since the information available conflicts in some extremely important points, we have reinvestigated the family. An autosomal recessive gene was probably responsible for the anomaly, rather than a dominant gene as proposed in the earlier hypothesis.

Six other families with at least one case of achiropody have been located in several Brazilian states, and it has been possible to study them. A full analysis of the problem, including the radiologic and dermatoglyphic aspects, will be published later in collaboration with Dr. R. A. Koehler and Dr. J. Lejeune.

Study of the seven families (including the first one) established the following facts: (1) All the abnormals have normal parents. (2) Three couples are consanguineous (one being uncle and niece, and the others first cousins), an incidence of consanguinity equal to 43% (3 out of 7). Taking the abnormals as the basis for calculation, 65% of them have consanguineous parents (13 out of 20). These frequencies are respectively 15 and 22 times those estimated for the Brazilian general population (cf. Freire-Maia, Am. J. Hum. Gen. *9*: 284–298). (3) The sex ratio among the abnormals is equal to .70 (14/20). Among their normal sibs it is .60 (21/35). The difference between the 2 values is not significant ($x^2 = .55$; $P > .30$). (4) The relation of 20 abnormals to 35 normals, verified among the children of the 7 families, agrees with the Mendelian ratio of 3:1, when duly corrected. The "sib method" leads to the ratio 13:35, for an expected ratio 12:36 ($x^2 = .11$; $P > .70$). According to the "*a priori* method," the expected number of abnormals is 15.65 ± 2.88. (5) The viability of the abnormals is not lower than that of their normal sibs, as measured by survival until 18 years of age. The frequency of the abnormals among the children born is 36% (20 out of 55), and increases to 43% among the children who attained at least 18 years of age (10 out of 23). The frequency of abnormals and normals at least 18 years old in comparison with the number born in each class equals .50 and .37, respectively. These differences, which actually favor the abnormals, are not significant ($x^2 = .35$ for the first comparison and .86 for the second one). At more advanced ages, the same conclusion seems true, but as the number of people decreases rapidly with age, any statistical analysis would be of dubious value. (6) None of the 20 abnormals had children. (7) Our data do not permit a reasonable estimate of

the prevalence of the anomaly in Brazilian populations, but it may be classed as very rare, taking as a standard the prevalences calculated up to the present for the majority of genetic anomalies.

Aspects (1) to (4) show that achiropody is due to an autosomal recessive gene. Although the viability of the abnormals does not seem to be different from that of the normals (5), inasmuch as our data do not show any case of reproduction (6), the frequency of the anomaly is probably being maintained in populations by recurrent mutation. As it is a very rare anomaly (7) the achiropody gene probably has an extremely low mutation rate. (This investigation was supported by grants from the Research Institute of the Faculty, the National Research Council of Brazil and the Rockefeller Foundation.)

FREIRE-MAIA, N., and A. FREIRE-MAIA. Effects of the Structure of First Cousin Marriages on the Coefficient of Inbreeding for Sex-linked Genes in Brazil. *Laboratory of Genetics, Faculty of Philosophy, University of Paraná, Curitiba, Paraná, Brazil.*

First cousin marriages may be subdivided into four types (A,B,C,D), according to the sex of the spouses' parents through whom consanguinity occurs. Type A represents relationship through two sisters ($f' = 3/16$); type B, through the wife's father and husband's mother ($f' = 1/8$); type C, through husband's father and wife's mother ($f' = 0$) and type D, through two brothers ($f' = 0$) (*cf.* Haldane and Moshinsky, Ann. Eug. *9*: 321–340; Wright, Ann. Eug. *15*: 323–354). In a population where the four types occur with equal frequencies, the mean f' (f'_m) would be equal to 5/64 or 0.078125, i.e., a value 25% higher than the coefficient f for autosomal genes (1/16 or 0.0625). Any social system favoring one or some of the above types will produce changes in the values of the population coefficient F' for sex linked genes.

Data obtained in "primitive" as well as in "modern" populations show that equality of the four types of cousin marriages ($\bar{A}=\bar{B}=\bar{C}=\bar{D}$) rarely occurs. In Europe and Japan, for instance, recent data show an excess of the classes A and B (system leading to an increase of F'); the opposite has been detected in India. Preliminary information obtained in two Brazilian populations showed that in one of them the distribution was different from that prevailing in Europe (Freire-Maia, in press, 1958). The analysis has been extended to seven other populations (Dioceses of Petrolina, Pe, and Pesqueira, Pe, in the Northeast; and Salvador, Ba, Barra, Ba, Belo Horizonte, MG, Campanha, MG, and Vitória, ES, in the East), and new data for São Paulo, SP, as well as those previously published for São Paulo and Curitiba, Pr (both of them in the South), have been included. These populations present differences in ethnic composition, economic basis, level of literacy, degree of isolation, etc. The data have been obtained through analysis of ecclesiastical files and so represent only Roman Catholic marriages, which are the great majority of all marriages.

An analysis of the data for the nine dioceses leads to the following main conclusions: (1) The samples from three dioceses (Pesqueira, Salvador, and Petrolina) show significant differences in the frequencies of the four types of first cousin marriages. As the differences reveal an excess of the types with $f' = 0$, the net result is a decreasing (respectively 2, 6, and 11%) in the values of f'_m. However, f'_m for the total data is still greater than the value of f (0.0625). (2) Type

D, with $f' = 0$, which occurs with a relatively low frequency in Europe and Japan, is one of the two most frequent in 10 out of the 11 samples analysed in the total, and in 8 out of the 9 recent ones. As a matter of fact, type D is the most frequent in 6 out of the recent 9. C + D is more frequent than A + B in 6 of them. The influence of these facts on the value of f'_m in the samples not referred to in the last paragraph is also small, the decreases being between 1 % and 12%. (3) Four samples (two from São Paulo, one from Belo Horizonte, 1922, and one from Campanha) present a distribution of the "European" type (A + B > C + D) and have, therefore, values of f'_m higher than those theoretically expected on the assumption of random mating. This increase is respectively 14 %, 8%, 16%, and 11%.

These variations in the values of f'_m are expected to have small effects on the population make-up if one uses as a standard the theoretical value 0.078125. Comparing, however, populations with relatively high values of f'_m (such as 0.08875, found in São Paulo) to populations characterized by relatively low values (such as 0.069137, found in Curitiba), somewhat larger effects are naturally to be expected. (This investigation was supported by grants from the Research Institute of the Faculty, the National Research Council of Brazil and the Rockefeller Foundation.)

FREIRE-MAIA, N. *See* FREIRE-MAIA, A., *et al.*

FRYE, SARA H. An Analysis of Minute Rearrangements of the Yellow Region in *Drosophila melanogaster*. *Indiana University, Bloomington, Ind., U.S.A.*

Minute rearrangements involving the left end of the X-chromosome of *Drosophila melanogaster*, having the scute-8 inversion, and the yellow-bodied exceptions were analysed genetically. Analysis confirmed that this region in this chromosome contains the following genes (among others) in the sequence here given: lethal J1+ (*1*J1+), lethal F+(*1*F+), yellow+(y+), achaete+(ac+), bobbed+(bb+). Tabulation of the frequencies of the radiation-induced cases thus far analysed, the loci of the respective + (indicating the normal allele) and — (indicating the recessive mutant allele or deficiency) signs are represented by their position in the above given sequence: — — — — — (26), — — — — + (118), — — — + + (0), + — — — — (0), + + — — — (1), + — — — + (1), + — — + + (3), + + — — + (12), + + — + + (7).

In addition, there were 32 cases with lethals or detrimentals in the euchromatic region to the right of bb+ which are still under analysis. Of these 32, 18 are — — — — — *1*, 11 are — — — — + *1*, and 3 are + — — — + *1*.

Of the 173 cases involving losses of tested loci to the left of and including yellow+, fifteen have been found to be capped by the right end of the II (8) or IV (7) (the only ends available for more precise testing). Of these 15 capped X-chromosomes, all are ac−, 6 are bb+ and 9 are bb−.

There were 3 spontaneous minute rearrangements, all were — — — — +.

The frequency of all exceptions combined are for 4000 r in relation to total count 126/41,310 and 1000 r 148/180,942 and spontaneous 7/263,694. Distribution of these exceptions in relation to dose will be presented.

(Work supported by a grant to H. J. Muller and associates from U.S. Atomic Energy Commission, Contract AT(11–1)—195.)

FUJII, KENJI. *See* YUASA, AKIRA.

FULLER, J. L., and J. P. SCOTT. A Ten-Year Experiment on Heredity and Behavior in Dogs. *Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.*

A wide variety of behavioral tests were administered to dogs of five pure breeds. Breed differences were demonstrated on most measures, whether of emotionality, learning, or specific behavior patterns. Hybrids between basenji (the African barkless dog) and cocker spaniels were also studied in order to determine genetic mechanisms. A demonstration will illustrate the plan of the experiment and summarize the results of selected tests.

FULLER, JOHN L. *See* SCOTT, J. P.

FUNG, SUI-TONG, JANICE STADLER, and JOHN W. GOWEN. Sex Differentiation as Affected by Major Genes and Gene Dosages. *Genetics Department, Iowa State College, Ames, Iowa, U.S.A.*

In *Drosophila*, adult sex is a consequence of integration of four and likely more cell systems. The sex combs are a part of the anteriorly located first leg discs, the gonads are mid-posterior, the genital disc is the last abdominal segment and body sizes are inclusive of whole animals. Cells susceptible to specific sex development are located in widely separated body regions. Sex mosaics show that the embryological field is under local control. Duplication of male or female secondary sexual systems in single individuals occasionally appears. In rare cases a full male reproductive system including testis and full reproductive tract may accompany a complete female tract including ovary. With polyploidy Bridges observed some six major distinguishable types; male extremes X + 3A and X + 2A, intersexes 2X + 3A and female extremes 2X + 2A; 3X + 2A and 3X + 3A. Female-influencing genes of the X chromosomes were held to balance male-influencing genes of the autosomes in evolving these types. Possible deficiencies in technique have thus far prevented demonstration of specific chromosome regions in the wild types which play specific roles in these reactions. Mutation, however, has shown that male-influencing regions exist in the autosomes in *D. virilis* (Lebedeff, 1939, and Newby 1942); in *D. melanogaster* (Gowen, 1942; Sturtevant, 1945; and others). These autosomal genes convert females into specific types of males or intersexuals. Melanogaster genes form an allelic series, F_1 progeny showing specific types traceable to the joint actions of both mutants. With polyploidy some ten types are known. They are not mosaics in time. Gene *Hr* causes the joint appearance of both male and female organs developed from the genital disc. Sturtevant's *tra* when homozygous retains the female body size while causing the gonads, genital disc and sex combs to develop fully male type. Sex comb variation in *Hr* types have characteristic variances similar to other characters. Contributions of dominance and epistacy as they operate in the embryological fields susceptible to sexual development are measurable and will be discussed. (This work has received assistance from Contract No. AT(11–1)107 from the Atomic Energy Commission.)

FURUHATA, TANEMOTO, and NORIO HAYASHI. Distribution and Inheritance of the Ear-Wax Type. *Department of Legal Medicine, Tokyo Medical and Dental University, Tokyo, Japan.*

It has been recognized that there are two types of ear-wax among human beings; one is gray, brittle and dry, the other blackish brown, yellowish brown, sticky and wet. The former is called the dry type and the latter is called the wet type. These two types are considered to be normal hereditary traits.

The authors have investigated the distribution of ear-wax type in 3,403 individuals in the Tokyo metropolitan area and found that the wet type occurred at a rate of 15.52%. This rate is somewhat different from previous reports.

According to the previous investigators this difference is due to differences of age and of areas tested for distribution.

The present investigation, in some cases, shows different rate of the frequency of each type by the determination between the two types of ear-wax.

The authors have investigated 950 families and confirmed that the wet type of ear-wax is inherited as the Mendelian dominant. This classification of ear-wax can be used in the analysis for cases of disputed paternity.

GAJEWSKI, W. The Relation between Age of Geographical Disjunction and the Degree of Differentiation in *Geum hispidum* Fr. *Botanic Gardens, University of Warsaw, Warsaw, Poland.*

Geum hispidum Fr. was discovered by Fries on the southeastern coast of Sweden. Plants similar to *G. hispidum*, found in Spain, were described as another species *G. albarraciense* Pau. On the Balkan Peninsula another species *Geum molle* Vis. et Panc. is known which is closely related to *G. hispidum*. We have thus three populations of plants morphologically closely related but geographically widely separated. The disjunction between the Balkan and Spanish populations is probably old, dating from the late Tertiary, whereas that between the Spanish and Swedish populations must be very new as the southeastern coasts of Sweden emerged from the sea only late in postglacial times. *G. hispidum* was probably introduced by man from Spain into Sweden. It is thus possible to compare the cytogenetic relationships among plants from these three populations in relation to the duration of the isolation period.

Plants from these populations were grown in Warsaw and their cytogenetic and morphological differences were analysed. All plants have 42 somatic chromosomes. The chromosomes are homologous, but in hybrids between *G. molle* and *G. hispidum* few univalents were found. The morphological differences between *G. molle* and *G. hispidum* are small but distinct. Those between Swedish and Spanish *G. hispidum* are barely perceptible. F_1 hybrids are intermediate and in F_2 generations the parental traits show continuous segregation. The more sensitive test for differentiation was hybrid fertility. The pollen and seed fertilities of the hybrids *G. molle* $\times$ *G. hispidum* were lowered to about 50% level, whereas in hybrids between Swedish and Spanish plants the pollen fertility was quite normal and seed fertility decreased about 15%. It may be concluded that in older disjunction the differentiation has proceeded to a stage which can be accepted as one of species, whereas in the more recent disjunction the differences are at the most of the variety rank.

GALINSKY, IRVING. Abnormal Chromosome Movement Resulting from the Realignment of the Polar Forces. *Biochemical Research Foundation, Newark, Delaware, U.S.A.*

The effects of "mitotic poisons" upon the polar forces or centers in the cell have generally been obscured by their more striking effects of the inhibition of the spindle and failure of the chromosomes to move to the poles. In normal mitosis there is a centralization of forces at the two poles and these are responsible for the equal distribution of anaphase chromosomes to the poles. "Mitotic poisons" as simple as inorganic phosphate when used in appropriate concentrations upset the balance of polar forces and result in abnormal distribution of anaphase chromosomes. The strength of the poles is changed to a remarkable degree and this is manifested by the proportion of anaphase chromosomes which move to the poles. A single pole may be so weak that it attracts only a few chromosomes or so strong that all chromosomes move towards it. The movement of the poles themselves can be changed and instead of moving to opposite ends of the cells, the poles may remain very close together.

A most unusual phenomenon occurs after the breakdown of the nuclear membrane. The spindle does not appear to function and the metaphase chromosomes are not oriented on an equatorial plate. After the kinetochore splits, the spindle may begin to function and the anaphase chromosomes may move to the poles. The chromosomes move at different rates and some may begin their movement after the others have already reached the poles.

The wide range of abnormal chromosome distributions which occur during phosphate treatment indicates that a number of phases are involved in the formation and action of the spindle apparatus upon which the phosphate can act. The earlier the stage of mitosis present when the phosphate acts, the greater the disruption of the mitotic processes.

GANI, RUTH. *See* FRANKEL, O. H., *et al.*

GARBER, EDWARD D. The Genus *Collinsia*: Cytotaxonomy and Speciation. *Department of Botany, University of Chicago, Chicago, Ill., U.S.A.*

The genus *Collinsia* Nutt. (Scrophulariaceae) includes twenty-one recognized species, mostly restricted to the Pacific coast states. The genus is divided into two major groups, depending on whether the flower pedicel is longer than or as long as to shorter than the calyx. The basic chromosome number is seven. Only a single polyploid species, *C. torreyi* ($2n = 42$) has been found; three species have not yet been studied. Seven species are included in the group with short pedicels, five species having a lower mean number of chiasmata per bivalent at metaphase I compared with either of the other two species in this group or with the species in the other group. It has been possible to detect subgroups of one or more species on the basis of morphology, crossability between species, and the cytology of interspecific hybrids. Within a subgroup interspecific hybrids yield only bivalents; between subgroups interspecific hybrids yield such aberrations as heterozygous reciprocal translocations and heterozygous paracentric inversions. The former hybrids have greatly reduced fertility; the latter are completely sterile. The

following working hypothesis for speciation in *Collinsia* has emerged: (1) chromosomal repatterning, followed by (2) mutation, and (3) much less significant, polyploidy. (Supported by the National Science Foundation and the Abbott Memorial Fund, The University of Chicago.)

GARTLER, STANLEY M. An Investigation into the Biochemical Genetics of B-aminoisobutyric Aciduria. *Division of Medical Genetics, School of Medicine, University of Washington, Seattle, Wash., U.S.A.*

This paper reports an investigation into the metabolic mechanism by which a genetic difference results in differential excretion of B-aminoisobutyric acid (BAIB). Two general hypotheses are examined in this respect: (1) differential renal function, and (2) differential thymine metabolism, a known precursor of BAIB. The data presented show that BAIB is not readsorbed by the renal tubules in either genetically determined high or low excretors of BAIB, which disposes of the renal hypothesis. Differential thymine metabolism is examined from three viewpoints: differential thymine synthesis, differential reductive catabolism of thymine, and differential metabolism of thymine via an alternative (e.g., oxidative) but unspecified pathway. The combined data strongly support the hypothesis that differential metabolism of thymine via an alternative pathway is the underlying factor in B-aminoisobutyric Aciduria, and that this system indirectly gives rise to the observed variation in urinary BAIB excretion.

GAWRONSKA, EVA M. S. *See* HSIA, DAVID YI-YUNG.

GERALD, P. S., and L. K. DIAMOND. Evidence for an Etiologic Differentiation of Dominantly and Recessively Transmitted Methemoglobinemic Cyanosis. *Children's Medical Center and Department of Pediatrics, Harvard Medical School, Boston, Mass., U.S.A.*

A series of experiments have been conducted on the blood of a representative member of a family exhibiting dominantly transmitted methemoglobinemic cyanosis. Electrophoresis of the oxidized hemoglobin was successfully used to separate the hemoglobin into a normal and an abnormal fraction. The abnormal fraction was found to have spectroscopic properties resembling those described by Hörlein and Weber in their investigation of a cyanotic condition likewise transmitted as a dominant characteristic. Since this abnormal hemoglobin differs from the normal in the globin portion of the molecule, its inclusion among the group of abnormal hemoglobins is justified and the designation Hgb M has been adopted. The dominant mode of transmission is thus in accordance with the common abnormal hemoglobin syndromes. The presence of Hgb M may be suspected in cases of hereditary cyanosis in which excessive amounts of reduced hemoglobin are absent and there is difficulty in the detection of methemoglobin by spectroscopic or chemical tests. A review of the recorded cases indicates that most instances of dominantly transmitted methemoglominemic cyanosis can be attributed to Hgb M disease. The recessively transmitted forms on the other hand have been associated with spectro-

scopically normal methemoglobin. The confusion surrounding the inheritance of methemoglobinemic cyanosis undoubtedly results from the existence of at least two etiologically distinct forms of the disease, (*a*) that associated with normal methemoglobin, and (*b*) that associated with the methemoglobin form of Hgb M.

GERASSIMOVA-NAVASHINA, HELEN. *Botanical Institute of the Academy of Sciences of the USSR, Leningrad, U.S.S.R.*

Mitotic Hypothesis of Double Fertilization in Angiosperms.

The greatest riddle of Double Fertilization consists in the fact that the two sperms, after having entered the embryo sac together, subsequently separate, moving in diametrically opposite directions; each of them penetrates into one of the two female cells (ovule and the central cell) and finally unites with its nucleus. It has been shown that this phenomenon cannot be attributed to cytoplasmic currents carrying the sperms but must rather be due to the very nature of the sperms themselves as well as to their interaction with the peculiar surroundings existing in the female sphere.

Careful studies made by the author have convinced her that the process of fertilization is governed by certain basic principles inherent in any living cell. These principles primarily concern movements of chromatids and sister nuclei at mitosis as well as movement and localization of nuclei under varying conditions arising in cells without mitosis. It appears therefore that the fertilization process may be analyzed only on the basis of a study of the development of the reproductive elements. The female sex cells attain a state of a profound mitotic rest and may be normally brought out of it only by the influence of the male fertilizing system. The sperms, on the other hand, owing to peculiar developmental conditions, remain in a state of incompleted mitosis (telophase) and cannot accomplish their developmental cycle without the female cells, the final resting stage being attained as late as in the zygote. The very movement of the sperms may be interpreted as mutual repulsion of the two sister nuclei—a phenomenon of the sort invariably observed in any mitosis; it is especially pronounced during spermatogenesis. The reason for the oriented character of this repulsion is seen by the author in the fact that the pair of sperms is delivered by the pollen tube just in the region between the two female cells in which, owing to an interaction of their nuclei, there exists a sort of cytoplasmic polarization (which is not unlike a secondary spindle or perhaps simply represents a relic of the preceding mitotic activity which produced the two female sister cells in question). This cytoplasmic orientation directs the motion of the sperms towards the opposite female cells. After having entered a female cell, the sperm, like any nucleus, is drifted to the dynamic centre of the said cell where the female nucleus is already found. Here occurs an interaction of the sperm with the female cell; this process results in resting condition of the sperm (formation of a nucleolus and loss of deoxyribonucleic acid), i.e., in the completion of its mitotic cycle under the influence of the resting female cell. The contents of the pollen tube in its turn brings the female cell out of rest and induces the zygotic mitosis, i.e., the initiation of a new ontogenesis.

One may thus assume that double fertilization rests upon mitosis which becomes singularly distributed in space and time and assumes various forms according to specific conditions influencing the development of the female and male sex elements. The development of the zygote may be interpreted therefore as a solution of con-

troversy created by the contrasting properties of the elements of opposed sexes assumed by them owing to the profound differences of their developmental conditions.

GERSHOWITZ, HENRY, and LEE E. SCHACHT. An Examination of the Correlation between Human Blood Types and Potentially Fatal Diseases of Children. *Department of Human Genetics, University of Michigan, Ann Arbor, Mich., U.S.A.*

A large body of evidence has arisen in recent years purporting to demonstrate the possible physiological effects of the genes determining the blood groups; more specifically, the ABO blood groups. The physiological correlations thus far demonstrated have, for the most part, been found in adults who are either in or past their reproductive years and do not appear to provide an adequate explanation for the existing blood group polymorphism. This study was devised to determine whether any correlations exist between blood groups and certain fatal or potentially fatal diseases of children. These diseases constitute a possible selective force which has not been sufficiently explored.

Children ranging in age from birth to 14 years, ascertained through the University Hospital and Children's Hospital of Michigan in Detroit, were selected as propositi on the basis of the following diagnoses: rheumatic fever, nephritis and nephrosis, leukemia, meningococcemia, congenital heart disease, hydrocephalus, and hemolytic anemia. Blood and saliva samples were obtained from all propositi, full sibs, and parents. This report concerns the results so far obtained on three of the study diseases; nephritis and nephrosis (55 families), leukemia (56 families), and congenital heart disease (42 families). As yet, no deviations from the normal population frequencies have been found among the propositi for any of the seven blood group systems studied. The results of the sib comparisons will also be presented.

(This project was supported, in part, by Grant AT(11–1)–405 from the U.S. Atomic Energy Commission.)

GIBBONS, I. R. Observations on the Fine Structure of Nuclei and Chromosomes in Locust Testis. *Cavendish Laboratory, Cambridge, England.*

Electron microscopic studies on thin sections of locust testis have not yet been able to elucidate all the changes of chromosomal organization that occur during the division cycles of mitosis and meiosis. However, at certain stages of development characteristic fine structure has been observed in chromosomes. The fine structure that has been observed in chromosomes of meiotic prophase, and that in nuclei of spermatids will be briefly described in this paper. Some evidence (Gibbons and Bradfield, Biochim. Biophys. Acta *22*: 506) suggests that further improvements in preparative techniques will be needed for a more complete elucidation of chromosomal fine structure.

Sections of locust spermatocytes show that during the early to middle diplotene stages of meiotic prophase the chromosomes contain a pair of dense fibrils, each about 250Å in diameter, running parallel to the long axis of the chromosome. These fibrils usually appear to run parallel to each other and 700Å apart (centre to centre); they have occasionally been observed twisted around each other in a

very loose spiral. In some preparations there appears to be a third fibril running midway between the two fibrils described above; this central fibril is thinner (about 100Å in diameter), and less well-defined than the outer pair. All of these fibrils taken together form only a very small part (of the order of 0.5% by volume) of the chromosomal substance and their relationship to the rest of the chromosome remains obscure.

In the nuclei of locust spermatids the whole of the chromatin becomes oriented into a large number of closely packed, elongated, narrow tubes that run parallel to each other and to the long axis of the spermatid (Gibbons and Bradfield, J. Biophys. Biochim. Cytol. *3*: 133). These tubes have a dense wall about 60Å thick; their over-all size is variable and decreases as the spermatid matures. In very late spermatids and mature sperm this oriented structure is no longer visible in electron micrographs—perhaps because it has become too closely packed. The appearance of the complex tubular structure in the chromatin of the nuclei can be correlated with the appearance of bi-refringence in the nuclei (Pattri, Zeit. Zellforsch. *16*: 723) and with the change in the chemical nature of the protein component of the nucleoprotein (Felix *et al.*, Progress in Biophysics *6*: 2) that occur during the formation of the mature sperm.

GILES, NORMAN H. *See* CHU, ERNEST H. Y.

GILMORE, L. O. *See* FECHHEIMER, N. S.

GINOZA, W. *See* SIEGEL, ALBERT, *et al.*

GLASS, BENTLEY, and L. E. METTLER. The Oxygen Effect in Respect to Point Mutations in *Drosophila melanogaster*. *Department of Biology, Johns Hopkins University, Baltimore, Maryland, U.S.A.*

Baker and Edington (1952) demonstrated that the frequency of sex-linked recessive lethals induced by X-rays in *Drosophila melanogaster* varies with the oxygen concentration at treatment, particularly at concentrations below that of air. At a dose of 4000 r about a doubling of the frequency of lethals was obtained. It is generally recognized, however, that a certain proportion of X-ray induced lethals are associated with chromosome breakage; and since essentially all other evidence relating to the oxygen effect in various organisms has applied to the frequency of chromosome breakage and reunion, it has remained uncertain whether or not the oxygen effect applies to point mutations.

Larvae 80 hours old (total age 120 hr.) possess germ cells in pre-meiotic stages only, and the frequency of chromosome aberrations recoverable from such cells is very low in the male germ line and seems to be zero in the female germ line. A representative sample of recessive lethals induced in irradiated 80-hour-old larvae of both sexes was examined cytologically. Of 35 lethals induced in male germ cells, 1 was in a chromosome carrying 3 breaks; no other lethal was associated

with any detectable breakage in the X-chromosome. Of 31 sex-linked lethals induced in 80-hour-old female larvae, none showed any chromosome breakage. Minutes, which frequently arise in connection with small deficiencies, are unobtainable from X-ray treatment of larval females.

Larvae 80 hours old were irradiated (2,000 r) in nitrogen, air, and pure oxygen. Although no significant difference was found between those irradiated in air and in oxygen, there was a significantly lower frequency in both sexes when irradiated in nitrogen than when irradiated in air. The frequency of lethals was consistently lower in the female series than in the male series; but the magnitude of the oxygen effect (approximately a doubling in frequency) was very similar in both. It may be concluded that the oxygen effect applies to point mutations as well as to chromosome breakage and reunion.

GLASSMAN, E., J. L. HUBBY, and H. K. MITCHELL. Maternal Effect of *ma-1*$^+$ in *Drosophila melanogaster*. *Department of Biochemistry, City of Hope Medical Center, Duarte, Calif.; Zoology Department, University of Texas, Austin, Texas;* and *Biology Department, California Institute of Technology, Pasadena, Calif., U.S.A.*

Previous work (Genetics *42*: 372) has shown that maroon-like (*ma-1*) and rosy (*ry*) eye color mutants of *Drosophila melanogaster* are deficient in xanthine dehydrogenase. Consequently, there is an accumulation of the substrates of this enzyme (hypoxanthine and 2-amino-4-hydroxypteridine), while the products formed from these compounds (uric acid and isoxanthopterin, respectively) have not been detected in these strains. Recent studies have shown that the genetically *ma-1* progeny from *ma-1*$^+$/*ma-1* or *ma-1*$^+$:= (attached-X) females are unexpectedly wild type in eye color; *ry*$^+$/*ry* females do not produce such "wild-type" offspring. Further studies have shown that these "wild-type" progeny are due to a maternal effect (pre-determination) of *ma-1*$^+$. However, only flies which emerge in the first 6 to 10 days of hatching show this effect; those which emerge later in the bottle have the typical *ma–1* eye color. This is due to a change in the food and not to the age of the female parent, since old females transferred periodically to new food will have maternally affected progeny in each new bottle. Biochemical studies have shown that in addition to increased amounts of red eye pigment, the maternally affected offspring have traces of isoxanthopterin and uric acid (the enzyme products), and do not accumulate as much hypoxanthine and 2-amino-4-hydroxypteridine (the enzyme substrates) as normal *ma–1* flies. This could indicate that small amounts of the enzyme have been passed through the egg, but no enzyme activity in extracts of these flies has been detected. Most maternal effects of this type affect processes in the egg, but the effect here seems to be much later.

GLAVINITCH, ROUJITZA. A, Transformation des blés d'hiver en blés de printemps héréditaires; B, Changement de l'hérédité chez le cotonnier. *Faculté Forestière, Belgrade, Yougoslavie.*

(A) D'après la théorie de développement par stades de T. D. Lisenko nous avons réussi, au cours de la période allant de 1947 jusqu'à 1950, par le changement

du complexe des facteurs extérieurs (températures, lumière) à transformer la variété de blé d'hiver héréditaire "Kooperatorka" en variété de blé de printemps héréditaire. (B) Au cours de la période allant de 1949 à 1954 nous avons réussi, par le changement des conditions extérieurs (les températures basses, etc.), à changer l'hérédité de la variété de cotonnier Big-Boll (de l'Amérique du Nord) à longue période de végétation et à obtenir une variété nouvelle "Makedonka," qui donne des fibres de qualité, a une courte période de végétation, et réussit bien dans les régions à cotonnier de Yougoslavie.

GLENK, H. O. *See* SCHWEMMLE, JULIUS, *et al.*

GOLDSCHMIDT, ELISABETH. Cryptic Genetic Factors Changing Pterine Concentrations of *Drosophila* Eyes. *Department of Zoology, The Hebrew University, Jerusalem, Israel.*

A stock of *Drosophila melanogaster* homozygous for the ey^2 gene differs in the concentrations of several pterines from the normal laboratory strains Berlin and Canton S. The fluorescent substances affected include F1 3 (isoxanthopterine), F1 4 (= F1 4A : 2-amino-4hydroxy-6-(1′,2′-dihydroxypropyl)-pteridine and F1 4B : 2-amino-4hydroxypteridine), F1 5 (probably 2-amino-4-hydroxy-7, 8-dihydro-8-lactylpteridine-6-carboxylic acid) and F1 7. (Identification of pterines based on Forrest and Mitchell, 1955, J. Am. Chem. Soc.: 77, and on Viscontini *et al.*, 1955, a, b, c, Helvet. chim. Acta: 38.) For convenience the changed pattern was characterized by the altered ratio of the fluorescent spots F1 5/ F1 4 (A & B), calculated from their fluorometric values after two-dimensional development of squashed heads and subsequent elution in water. With the colour filters utilized (366μm. and 440μm.) this ratio is always larger than 1 (1.5—2.5) in the normal strains and invariably below 1 (0.5—0.8) in the ey^2 under investigation. The "altered ratio" behaves autonomously in ey^2 eyes transplanted into normal hosts.

It could, however, be shown that the altered F1 5 / F1 4 ratio is by no means characteristic of all flies homozygous for the ey^2 gene. In an attempt to transfer the fourth chromosome of the *ey* stock to a background of chromosomes of the Berlin strain, *ey* flies were backcrossed for 54 generations to Berlin. The resulting *ey* flies show a partially normalized F1 5/ F1 4 ratio, approaching one and differing significantly from both that of Berlin and that of the original *ey* stock. A more complete normalization of the ey^2 chromatogram was achieved by transferring the fourth chromosome of the *ey* strain to a background of Berlin chromosomes in a system of crosses utilizing the well-known marked "balancer" chromosomes.

In order to identify the chromosome(s) responsible for the "altered ratio" effect, a number of stocks were synthesized carrying different combinations of chromosomes from the original *ey* and the Berlin stocks. After elimination of all "marked chromosomes" whose presence might affect the pterine concentrations, these stocks were analysed chromatographically. The results indicate that the "altered ratio" depends chiefly on the second and fourth chromosomes of the *ey* stock. Since the second chromosome does not produce any other phenotypic deviations from normal, its effect on pterine concentrations may be considered as "cryptic."

In view of the increasing utilization of the pterine patterns for the construction of taxonomic keys, it should be realized that cryptic genetic variability may give rise to striking intraspecific differences in the concentrations of these substances.

GOLOVINSKAYA, K. A. *See* PROKOFIEVA-BELGOVSKAYA, ALEXANDRA, *et al.*

GOODGAL, SOL H. Expression and Segregation in *Hemophilus influenzae* Transformations. *The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland, U.S.A.*

When *Hemophilus* cells are exposed to DNA derived from a variant strain, a fraction of these cells are transformed to the variant, and this number is directly proportional to the amount of DNA taken up irreversibly by the cells. Furthermore, it has been shown that transformations follow a single hit process, presumably due to the interaction between a particular molecule of DNA and the host cell.

Once a cell has taken up a molecule of DNA, metabolic activity is required before the cell can express the factor inducing transformation. Chloramphenicol, an inhibitor of protein synthesis in concentrations of 20γ/ml., blocks the development of competence in cells but does not prevent the irreversible uptake of DNA by cells that had previously developed competence. It also prevents expression of a transforming factor in cells, but if the inhibitor is removed before the cells lose viability, there is no loss in number of transformed cells.

In agreement with Alexander and Leidy, and Shaeffer, we have also shown that cells which undergo transformation for streptomycin resistance develop interesting resistance to the antibiotic. Some level of resistance develops within 30 minutes after uptake of the DNA molecule. If these cells are plated in the absence of the selecting agent and the clone analysed, the original cell divides normally giving rise to streptomycin sensitive progeny as well as streptomycin resistant progeny. However, a pure streptomycin resistant clone does not occur for 90 minutes (three generations) after DNA uptake. Linked transformations for streptomycin and cathomycin exhibit similar behaviour with no evidence of segregation of the single characteristics in the 23 clones analysed. Clonal analysis of unlinked double transformations shows that about one-fourth of the clones give single segregants. Possible mechanisms for these results will be discussed.

GOODMAN, B. L. *See* JAAP, R. GEORGE.

GOWANS, CHARLES S. Genetic Investigations on *Chlamydomonas eugametos*. *Department of Botany, University of Missouri, Columbia, Mo., U.S.A.*

Genetic studies have been made with the unicellular green alga *Chlamydomonas eugametos,* starting with wild-type stocks originating from F. Moewus.

By a replication technique, following ultraviolet irradiation 39 nutritional mutants were obtained, including strains requiring special carbon-sources, nicotin-

amide, *para*-aminobenzoic acid, thiamine and purines. We also obtained 25 morphological and physiological mutants characterized by loss or paralysis of flagella, twinning, altered pigmentation, requirement for high osmotic pressure, and streptomycin resistance.

Studies on crossing-over have been done almost exclusively by means of tetrads. Some 3,000 segregants from over 800 zygotes have been characterized in crosses where from 1 to 5 genes were segregating. Equations for computing genecentromere distances have been applied, and eleven genecentromere distances have been computed. Normal linkage has not been detected, but cases of "negative linkage" (a significant excess of non-parental ditype tetrads) have been obtained. Tetratype frequencies in excess of two-thirds have been found. Preliminary studies on the relationship between temperature and crossing-over frequency indicate that it is not a simple linear one.

Hybrid zygotes from *C. eugametos* $\times$ *C. moewusii* (from R. A. Lewin) gave good germination, and the progeny showed recombination for five segregating genes, making doubtful the status of these two as separate species.

No evidence has been found to support Moewus' report that lower temperature inhibits crossing-over at the 4-strand stage, or that sex determination involves non-allelic genes. Moreover, tetratype segregations have been observed for unlinked genes, contrary to Moewus' published data.

GOWEN, JOHN W., and MAGNHILD UMAERUS. Biological Recovery from Radiation Effects as Related to Genetics. *Genetics Department, Iowa State College, Ames, Iowa, U.S.A.*

Radiation exposure, to many people, means irretrievable injury to health, vigor, life span, reproductive cells and future progenies. For genes, doubling the roentgen dose doubles the mutation rate; thereby increasing the dangers to subsequent generations. These conclusions seem incompatible with life having a continuum of irradiation with highly penetrating energy of chemical and outer space origins over millions of years.

When mice are irradiated there is an acute effect on life span followed by partial recovery. The authors have examined populations of *Drosophila* germ cells and find that recovery is expressed in several ways. Cells of the *Drosophila* germ tracts were exposed to multiples of 2,000 r given on 0 days after emergence and at 14, 28 and 42 days thereafter. Eight days immediately following the first irradiation the percentages of sex-linked lethal mutations were: no radiation, 0.0; 2,000 r, 3.6; 4,000 r, 10.3; 6,000 r, 13.5; and 8,000 r, 18.3. A 14-day interval between irradiation and the germ cell sample had reduced the initial lethal percentages to one-fifth, a 28-day interval to one-seventh, a 42-day interval to one-eleventh. The potential germ cell population has undergone biological improvement.

To allow time for repair irradiations were spaced: 2,000 r at 0 day; 2,000 r at 14; 2,000 r at 28; and 2,000 r at 42 days, giving an accumulated series of 2,000, 4,000, 6,000 and 8,000 roentgens to each male. Sperm from these periods showed mutation rates of 4.9, 5.2, 5.9 and 10.7% as contrasted with 3.6, 10.3, 13.5 and 18.3% where the corresponding irradiation dosages were received in single periods. The populations of repeatedly irradiated sperm recovered most of their normal characteristics before the following irradiation again raised the mutation percentages. Mutations in early germ cell lines appear to increase somewhat the

observed lethals in the progenies of the 42-day males receiving high irradiation dosages. Data on visible mutations, survival of offspring in first and second generations bear on this problem and may be presented. Biological recovery has significance in clearing radiation damage from both the soma and populations of germ cells. (This work has received assistance from Contract No. AT(11-1)107 from the Atomic Energy Commission.)

GOWEN, JOHN W. *See* BRYAN, JOHN H. D.; FUNG, SUI-TONG, *et al.*; STADLER, JANICE.

GOWER, JOSEPHINE S. *See* RUSSELL, W. L.

GRAF, G. E. Paper Chromatographic Analysis of Heterozygotes in *Drosophila melanogaster*. *University of Zürich, Zürich, Switzerland.*

The fluorescent patterns, due primarily to pteridines, have been studied quantitatively in *Drosophila melanogaster* heterozygous for a number of genes generally regarded as recessive. Heads of three day old imagos were chromatographed as described by Hadorn and Mitchell and fluorescence measurements were made directly on the paper. Some eye colour mutants, though not all, caused readily reproducible, quantitative changes in the content of fluorescent substances, but no qualitative differences were found. These changes were caused in a number of different wild-type lines and remained essentially the same after the mutant had been back-crossed to the wild-type line for a number of generations. It was further found that organisms heterozygous for two mutants often showed patterns whose deviations from wild type were difficult to explain on the basis of the action of the individual mutants. (This study was aided by a post-doctoral fellowship grant of the American Cancer Society.)

GRAHN, DOUGLAS, KATHERINE F. HAMILTON, and GEORGE A. SACHER. Genetic Variation in Chronic Radiation Mortality and the Reduction of Life Expectancy among Inbred Mouse Strains following Single Dose Whole-Body X-Irradiation. *Argonne National Laboratory, Lemont, Ill., U.S.A.*

Inbred mouse strains BALB/c, A/Jax, A/He, $C3H_f$/He and C57BL/6 were exposed at an average age of 85 days to acutely lethal single doses of 200 kvp X-rays. $LD_{50/30}$ values ranged from 500 r for strain BALB/c to 630 r for C57BL/6 (Grahn and Hamilton, Genetics *42*:189). Survivors of the acute syndrome were kept for an analysis of chronic radiation injury.

Significant differences in reduction of mean after expectation of life are observed between sexes and among strains within each sex. At a constant dose of 570 r, females show a 29.7 $\pm$ 1.0% reduction as compared to 22.0 $\pm$ 1.2% for males. Within sex, the reduction of life varies with strain from 22% to 41% for females, and from 16% to 30% for males. At their respective $LD_{50/30}$ doses, the strains also

express significant differences. This variation in the reduction of after-survival must be under genetic control, since it cannot be related to differences in either dosage or levels of acute injury.

Analysis of age specific mortality rates indicates that the life tables of most strains are similarly altered by single irradiations. The regression of log mortality rate on age is displaced to the left (or upward) of the control, but remains reasonably parallel to the latter, as noted in an earlier study (Sacher, Radiology *67*:250). Perturbations of this pattern are noted in strain C57BL/6, which has a high sensitivity to radiation leukemogenesis, and in strain BALB/c, which has a high infectious disease incidence.

An intriguing positive inter-strain relationship is detected between the magnitude of the acute $LD_{50/30}$ dose and the mean life expectancy of unirradiated mice. Small and variable sex differences in both parameters also tend to be directly proportional. While strain differences in reduction of life are not related to differences in either control survival or $LD_{50/30}$, the latter reflects the normal viability of the strain. (This work was performed under the auspices of the U. S. Atomic Energy Commission).

GRANT, WILLIAM F. Cytological Aspects of Sex Determination and Phylogenetic Trends in Dioecious Species of *Amaranthus*. *Department of Genetics, McGill University, Macdonald College P.O., Que., Canada.*

Cytological observations have been carried out on four dioecious species of *Amaranthus*, two of which were formerly included in the genus *Acnida*. The species studied are *A. arenicola, A. palmeri, A. tamariscinus* and *A. tuberculatus*. Three of these species have a diploid chromosome number of 32, while *A. palmeri* has a somatic chromosome number of 34. A single spontaneous triploid ($2n = 48$) female plant was found in collections of *A. tamariscinus* and a tetraploid ($2n = 64$) male plant in collections of *A. tuberculatus*. A fifth species, *A. australis*, has previously been reported as having 32 somatic chromosomes. The chromosome numbers for half the dioecious species of *Amaranthus* have now been determined.

Detailed observations on the dividing chromosomes of these species in mitosis, meiosis and in the first division of the nucleus in the pollen grain have been made and have failed to distinguish heteromorphic chromosomes which might be associated with sex determination.

It has not been possible to indicate phylogenetic trends based on the morphology of the chromosomes. The small size of the chromosomes has made detailed morphological studies impractical and there is no marked difference in absolute size of the chromosomes between species.

Since basic numbers of 16 and 17 are found in both monoecious (reported and unpublished observations) and dioecious species, it would seem that the aneuploid condition in *Amaranthus* arose early with subsequent divergent evolution.

GREB, RAYMOND. *See* MORGAN, WALTER C.

GREEN, G. J. *See* WELSH, J. N.

GREEN, M. M. On the Occurrence of Non-Homologous Pairing and Crossing-over in *Drosophila*. *Department of Genetics, University of California, Davis, Calif., U.S.A.*

The sporadic occurrence in *Drosophila* of tandem duplications exemplified by *Bar, Beadex-recessive* and the *Star* duplication can be accounted for by assuming that crossing-over occurs within paired, non-homologous segments of chromosomes. Presumably the products of these events should include deficiencies as well as duplications. Investigations of the crossing-over relations of the sex-linked, recessive white pseudo-alleles *apricot* (w^a) and *apricot-2* (w^{a2}) in *Drosophila melanogaster* uncovered genetic events which best fit an interpretation of non-homologous pairing and crossing-over occurring under genic control.

From homozygous w^a females made heterozygous for appropriate recessive marker genes to detect the occurrence of crossing-over as well as autosomal inversions *Curly* and *Ultrabithorax-130* two exceptional types have been recovered, each associated with a single crossover. Genetic evidence shows that one exception consists in a loss of either a portion of or the entire *white* region of the chromosome and a chromosome segment of undetermined length to the left of *white*. Supporting this conclusion, we find that the exceptional chromosomes produce male lethality, show pseudodominance when heterozygous for *w* mutants, cannot be covered by duplications known to cover the *white* segment but not extending beyond the left limits of *white* (e.g., *Dp* (1;3) w^{+5167}), and can be successfully covered by longer duplications covering *white* as well as gene loci to the left of the *white* (*Dp* (1;3) $w^{v},^{co}$). Analysis of the second exception demonstrates that it is a tandem duplication in which w^a together with a chromosome segment of undetermined length to its left is repeated. Phenotypically the w^a duplication is distinctly darker than w^a. From females heterozygous for the duplication and a wild-type chromosome, w^a has been recovered in association with unequal crossing-over.

Additional exceptions will be presented and the occurrence of the exceptional types will be discussed. It will be shown that these data furnish direct evidence that crossing-over is not invariably an event associated with allelic associations of homologous chromosomes.

GRELL, E. H. Genetics and Biochemistry of "red cell" in *Drosophila melanogaster*. *California Institute of Technology, Pasadena, Calif., U.S.A.*

The mutant phenotype of *Drosophila melanogaster*, "red cell," is characterized by the coloration of some of the free floating fat cells in the hemocoel with a reddish pigment related to the brown eye pigment (Jones and Lewis, 1957).

Genetic analysis reveals that a pair of complementary recessive genes produces the "red cell" phenotype. Both genes, *rc* (red cell) and *lys* (lysine), must be homozygous for the fat cells to be pigmented. Employing the markers Sternopleural, dachs and Jammed the following map of the five loci can be constructed from analysis of cross-over data: *Sp*–.9–*lys*–1.8–*d*–2.4–*rc*–4.5–*J*. If *Sp*'s standard map location of 22.0 is used, *lys* is 22.9 and *rc* is 27.1 map units from the left-most marker on the second chromosome.

No detectable phenotype is produced by *rc* without *lys*; but *lys* homozygotes, with or without *rc*, show an accumulation of the amino acid, lysine, when they are squashed on paper and chromatographed. Lysine is accumulated because *lys* probably blocks some step in its degradation. Eight hours after injection of C^{14} randomly

labelled 1-lysine into Canton-S and *lys* adult males, 30% of the recovered radioactivity is found in the respired CO_2 of the wild-type, but only 3% is found in the respired CO_2 of *lys* flies. A dicarboxylic acid, which becomes more highly labelled after injection of labelled lysine into Canton-S than after injection into *lys* flies is believed to be an intermediate in the degradation of lysine and to be formed subsequent to the step at which *lys* blocks the degradation pathway.

GRELL, RHODA F. Preferential Segregation of the Sex Chromosomes in XYY Males of *Drosophila melanogaster*. *California Institute of Technology, Pasadena, Calif., U.S.A.*

Data of Bridges (1916) and Stern (1929) have usually been interpreted as meaning that the segregation of the sex chromosomes in an XYY male of *Drosophila melanogaster* is non-preferential. If such is the case, the ratio of XY/Y to X/YY segregations is expected to be 2:1. In the present experiment, males which carry two normal Y chromosomes derived from a Canton-S stock as well as a normal X chromosome were mated to females with a dominant brown-variegated (bw^{VDel}) phenotype. The brown variegation, in this case, is completely suppressed in the presence of an extra Y chromosome. The 2,822 brown-variegated progeny from these matings fall into the following classes, 1,153 XXY females, 307 XX females, 1,155 XY males and 207 XYY males. The ratio of XXY:XX female progeny based on these data is 3.75:1 indicating preferential segregation. The corresponding ratio for male progeny is 5.6 XY to 1 XYY. This somewhat higher ratio may indicate a lowered viability for XYY males. Bridges' data consisting of 39 XXY and 15 XX daughters of XYY males are in fact statistically compatible with a 3.75:1 ratio.

GRIFFITHS, D. J., D. G. ROWLANDS, and W. T. H. PEREGRINE. Cytogenetic Inter-relationships of Certain Artificial and Natural Polyploid Species of *Avena*. *Welsh Plant Breeding Station, University College of Wales, Aberystwyth, Wales.*

One of the primary purposes of synthesizing new amphidiploids in cereal crops is to study the possibilities of introducing genetic factors controlling valuable economic characters from related diploid and tetraploid species and genera to varieties of agricultural importance.

In *Avena*, various experimental 6x amphidiploids have been produced through crossing tetraploid and diploid species followed by colchicine treatment of the resultant triploid hybrids. Representative plants from the A_2 and A_3 generations of the 6x amphidiploid developed from *A. barbata* × *A. strigosa* sub. sp. *hirtula* were crossed with the natural hexaploid species as well as with other amphidiploids and the data obtained on meiotic behaviour, seed fertility, and morphological characteristics of the F_1 hybrids are presented and discussed.

Hybrids derived from crosses between the amphidiploids and the natural species and those derived from amphidiploid × amphidiploid crosses revealed differences in chromosome pairing, seed fertility and in the dominance relationships of the mode of spikelet disarticulation, floret disjunction and degree of awning.

All hybrids between the amphidiploids and the natural hexaploids proved to be almost completely self-sterile whereas those obtained from crosses involving different

amphidiploids gave seed sets comparable with their parents. Bivalent formation in the former group of crosses was markedly lower than in the amphidiploid parent, the mean values being 7.8 bivalents compared with 14.34, and in the latter crosses was similar to that observed in parental plants, but with rather fewer univalents.

In contrast with the 6x amphidiploid $\times$ *A. sativa* hybrids in which separation of the spikelet from the peduncle by fracture was partially dominant over the shedding base, hybrids derived from the 6x amphidiploid and other amphidiploids with cultivated base type spikelets exhibited a complete reversal of the dominance relationships, the shedding base type being dominant over the normal base as found in the diploids and tetraploids.

GROSCH, DANIEL S. Somatic Cytology of the Wasp *Habrobracon. Department of Genetics, North Carolina State College, Raleigh, N.C., U.S.A.*

Multinuclear structural units are typical of the internal tissues of *Habrobracon* larvae. The multinucleate condition is attained by amitotic divisions resembling those of the macronucleus of ciliates. The nuclei of multinucleate units become highly polyploid during development. Tissues so comprised do not persist through metamorphosis. Adult tissues typically show uninucleate cells although certain nuclei enlarge greatly and are highly polyploid in the functional state. Trophocytes are good examples of such cells. Members of a group of these cells are structurally and functionally interrelated. Structural relations are seen as protoplasmic bridges. Functional relations are revealed by the staining reaction after the wasp has been exposed to radiation or a chemical agent. All members of the group react similarly. Thus, in both larvae and adults, the structural mechanism of secretory and elaborative physiological activity is more complex than merely a unit composed of a circumscribed mass of protoplasm containing a single nucleus.

GRUN, PAUL. Cytological Instability of Accessory Chromosomes from Native Populations of *Allium cernuum. Department of Botany and Plant Pathology, Pennsylvania State University, University Park, Pa., U.S.A.*

Telocentric fragment-sized euchromatic accessory chromosomes have become established in native populations of nodding onion, *Allium cernuum*, over an extensive area. Variant types, including larger accessories and an isochromosome form, are more locally restricted. Proportion of plants of fourteen separate populations from Pennsylvania and West Virginia containing accessories varies through a continuous series from 0% to 50%. There is no apparent relationship between geographical distribution or the local ecological situation and frequency of accessories in a population.

Telocentric accessories are highly unstable in mitotic divisions as reflected in variability of number in different cells within and between root tips and in different microsporocytes of each plant. By contrast, the isochromosome is mitotically stable. Analysis of second anaphase figures of meiosis shows that telocentric accessories are distributed as follows: they do not pair with one another or with the normal chromosomes, but drift to poles at random during first anaphase, few being lost to micronuclei; during the second anaphase they divide normally.

Allium cernuum accessories differ from those previously described in no single

character, but in the alignment of previously described types of variability. The cytogenetic resemblances and differences among known types of accessories will be discussed in relation to their evolutionary significance.

GURGEL, J. T. A. The Non-Homologous Associations of Centromeres and Knobs of Maize Chromosomes at Meiosis. *Escola Superior de Agricultura "Luiz de Queiroz," São Paulo, Brazil.*

In the present work the author deals with the non-homologous association of centromeres and knobs in maize, the two exceptions to two-by-two pairing of chromosomes at pachynema. This research was carried out in the inbred KYS and in 5 homozygous reciprocal translocations. It was shown that the number of centromere adhesions is a function of relative length for all the chromosomes with exception of chromosome 5, which is more frequently involved than expected. Also, it was found that in spite of not being significant for each case studied, chromosomes 1, 2, 6 and 10 are encountered less often in centromeric adhesions, and chromosomes 4, 7 and 8 oftener than one would expect. In considering knob adhesions, the frequencies found deviate largely from expectation. For both centromere and knob adhesions, it was found that the presence of the nucleolus attached to chromosome 6 tends to reduce the associations of this particular chromosome in pairs. The data found in this research for centromere, knob and terminal ends associations do not support the hypothesis that heterochromatin is responsible for these associations.

GYÖRFFY, B., and I. KÁLLAY. Discrimination between Genotypic and Phenotypic Variation of Streptomycin-Resistance in *Serratia marcescens*. *Institute of Genetics, Budapest, Hungary.*

Despite considerable experimental evidence that drug-resistance has a genetic origin, there is still some controversy concerning its development in bacterial populations. By the information-decoding receptor model we can distinguish between the origin of resistance as a potency of the genotype and its development as a secondary process expressed in the phenotype. Ignorance of this distinction resulted in a widespread confusion of the shift at the tail of phenotypic variation with genuine genetic changes. We designed our experiments to decide the issue by critically discriminating technique and to resolve the response of the individuals, not just the change in the population as a whole.

The indirect evidences we obtained for the genotypic variation in streptomycin-resistance are: discontinuous increase in serial transfers with small inocula, stable variants with low level of resistance, statistical demonstration of preformed clones and a good approximation to the statistical prediction of the expected distribution, and mutation rates similar to those observed in other bacteria. The range of the phenotypic expression of a genotype is broader than is assumed. Culturing or "training" performed in complex medium impressed changes leading to a shift in the limits of the phenotypic variation without altering the genotype, thus simulating a continuous increase in resistance. But the complex medium also increases the proportion of true resistants by favoring the phenotypic expression of newly mutated cells arising in the culture. By re-tests in differential medium the limits of the

phenotypic variation could be satisfactorily narrowed and the disturbing occurrence of overlap between physiological and genetic variation eliminated. This could also be proved in artificial reconstruction experiment with sensitive and phenotypically expressed resistant cells.

HAAS, FELIX L., and C. O. DOUDNEY. Relations between Nucleic Acid and Protein Syntheses in Ultraviolet-Induced Mutation in Bacteria. *University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas, U.S.A.*

A hypothesis has been advanced that nucleic acid precursors altered *in vivo* by ultraviolet (*UV*) constitute chemical intermediates in mutation (Haas and Doudney, Proc. Nat. Acad. Sci. *43*: 871–883). Increased UV-induced mutations in *Escherichia coli* strain B ("EMB-color" mutations) and in *E. coli* wp2 (reversion of tryptophan requirement) by pre-irradiation incubation in basal medium supplemented with RNA ribosides or purines and pyrimidines have led to this conclusion. Witkin's studies (Cold Spring Harbor Symposia Quant. Biol. *21*: 123–140) and ours indicate that mutation induction is related to post-irradiation supply of amino acid. Our studies also indicate that UV-induced mutation may depend on *post-irradiation* synthesis of nucleic acid from radiation-modified precursors, and probably involves concurrent protein synthesis.

Further studies (Doudney and Haas, Proc. Nat. Acad. Sci., in press, 1958) have shown that the amino acids are required for a process of post-irradiation "mutation stabilization." When irradiated cells are held under certain conditions limiting to amino acid or protein synthesis, there is immediate decline in induced mutation frequency. If amino acids are present, the mutations are subsequently "fixed" into the genetic composition of the cell.

The following has now been established regarding post-irradiation "mutation stabilization" and "mutation frequency decline": "mutation stabilization" requires a complex source of amino acids, and an energy source. Omission of one of these factors results in "mutation frequency decline." Deletion of phosphate from the medium, or dinitrophenol inhibition, prevents "mutation stabilization," but does not lead to "mutation frequency decline." By using 5-hydroxyuridine (an analogue blocking orotic acid conversion to uridine, thus preventing RNA synthesis) in the post-irradiation incubation medium it has been found that processes leading to RNA synthesis are involved in "mutation stabilization." The results suggest a possible intermediary role of RNA and protein synthesis in genetic DNA replication.

HABERLANDT, WALTER F. Genetic Aspects of Amyotrophic Lateral Sclerosis and Progressive Bulbar Paralysis. *Institut für Humangenetik der Universität Münster, Münster/Westf., Germany.*

Owing to the initiative of Prof. v. Verschuer, Director of the Institute of Human Genetics, University of Münster, and with the aid of a large staff of co-workers, a thorough registration of pathological traits and clinical syndromes (considered to be caused by mutant genes) is being carried out, in a population of about 2 million people, to supply data for statistical studies.

Two conditions investigated for the last two years are amyotrophic lateral sclerosis and progressive bulbar paralysis. A systematic search in neurologic

departments and mental hospitals of Westfalia revealed 251 index cases in a population of about 250,000 neuro-psychiatric patients observed during the last 30 years.

Our investigation is a family study (including a small twin series only). All living probands are given clinical examination and anthropological tests; catamnestic data are obtained from relatives of deceased index cases. The clinical results of our research are in good agreement with those reported by other authors.

The genetic analysis of our material may help to clear up inconsistent concepts based on earlier data. Of the cases re-examined so far 13.5% are hereditary. In our pedigrees an autosomal dominant gene with incomplete penetrance and varying expressivity can be demonstrated. Apart from this genetic basis for the manifestation of amyotrophies, environmental influences must be taken into consideration. The etiology of Charcot's disease could be explained by both constitutional and peristatic factors. Its pathogenetic mechanism is still unknown; it may be some metabolic defect. As for the sporadic cases (which comprise the great majority in all samples so far published), the role of mutations is discussed. Mutation rates of Charcot's disease have not yet been calculated.

HAGA, TUTOMU, and SHOZO NODA. Cytogenetic Population Structure of *Scilla scilloides* Druce: A Complex. *Cytogenetics Laboratory, Department of Biology, Kyushu University, Fukuoka, Japan.*

Karyotypes of the two basic complements A(x=8) and B(x=9) are clearly distinguishable in diploids and in polyploids of *Scilla scilloides* Druce. At meiosis, types of the constitution AA form 8_{II}, BB 9_{II}, BBB 9_{III}, ABB $9_{II}+8_{I}$, and AABB 17_{II}. Therefore, the two basic complements are genetically well differentiated from one another. Pairing $2_{III}+7_{II}+6_{I}$, observed in 1% of the PMCs in ABB, suggests a remote homological relationship between the two distinct basic complements.

The types of chromosome constitutions found in natural populations in the vicinity of Fukuoka, Japan, were as follows: BB, BBB, ABB, AABB, ABBB ($9_{III}+8_{I}$) and AABBB($9_{III}+8_{II}$). Each of the types is further classifiable into several subtypes with respect to the supernumerary chromosomes, structural rearrangement, for instance BB(9_{II}) and $BB^{T}(.4+7_{II})$ in the type BB. The type AA is known only from South Korea.

Natural population at a given locality consists of the types with constitutions related to one another: for example, BB(8), BB^{T}(30), and BBB(12) at Mitusawa; BB(16), BBB(21), ABB(39), AABB(14), and ABBB(34) at Nakabaru. Figures given in parentheses indicate the number of plants examined cytologically. Thus, it is clear that the compositions of the natural populations of this complex have been much complicated by hybridization and also by vegetative propagation of the sexually unstable or sterile types.

HAGEMANN, RUDOLF. Somatic Gene Conversion in the Tomato. *Institute for Research of Cultivated Plants, Gatersleben Kreis Aschersleben, Germany.*

A series of multiple alleles of a locus named sulfurea has been found. The homozygotes of sulfurea^{pura} alleles (*sulf^{pura1}*, *sulf^{pura2}*, etc.) have yellow cotyledons. They

die within a fortnight if not grafted on control stocks. The grafted *sulf*^{pura} plants form pure yellow leaves. The homozygotes of *sulfurea*^{variegata} (*sulf*^{vag}) are semilethal. The leaves of all grafted sulf^{vag} plants are green-white variegated. This variegation is largely due to modifications. Grafted *sulf*^{pura} and *sulf*^{vag} homozygotes are fertile. The heterozygous *sulf*$^{+}$*sulf* plants (*sulf* means *sulf*^{pura} or sulf^{vag}) have green cotyledons like those of normal *sulf*$^{+}$*sulf*$^{+}$ plants. In further development part of the heterozygotes shows a green-white variegation of foliage leaves. The *sulf* alleles isolated till now differ by the percentage of variegation they cause in heterozygous plants. Progenies of both green and variegated branches have been analysed. The variegation of the foliage leaves is caused by mutation of the normal allele *sulf*$^{+}$ to a recessive *sulf* allele. The white regions are of *sulf sulf* constitution. The *sulf*$^{+}$ allele is stable in homozygous pants. In heterozygous condition, however, mutations of *sulf*$^{+}$ to *sulf* occur under the influence of the *sulf* allele already present. Such a directed mutation, which has been termed "conversion" by H. Winkler (1930), occurs in somatic tissues of the shoot extending to generative tissues if the mutated areas take part in the formation of reproductive organs. Thus deviations from normal 3:1 segregation appear resulting in an increase of *sulf sulf* seedlings. In the present case, consequently, somatic conversion is concerned. Problems of conversion in sulfurea as well as relations between somatic and meiotic conversion will be discussed.

Hair, J. B. The Chromosomes of the Podocarpaceae. *Botany Division, Department of Scientific & Industrial Research, Christchurch, New Zealand.*

The chromosomes of representative species of the 7 genera of the Podocarpaceae are analysed and compared. All genera are diploid, with basic numbers as follows: *Phyllocladus*, 9; *Acmopyle*, 10; *Saxegothaea*, 12; *Pherosphaera*, 13; *Microcachrys*, 15; *Dacrydium*, 9–12, 15; *Podocarpus*, 10–13, (16), 17–19. Cytological evidence suggests that with certain exceptions these numbers fit an evolutionary series in which repetitive "centric fusions" reduce a primitive basic set of 20 acrocentric chromosomes ($2n = 40$) to a "saturated" level of 10 metacentrics only ($2n = 20$). At any point in this series the number of metacentric pairs in homozygotes corresponds to the number of fusions which have taken place. Of the 11 chromosome numbers expected in this scheme 8 (potentially 9) have so far been found, including 7 in *Podocarpus* alone.

The system proposed rests on the following observations: (1) close morphological correspondence between the idiograms of related species of adjacent basic numbers; (2) chromosome pairing in natural hybrids—the hybrid between *P. nivalis* ($x = 19$) and *P. hallii* ($x = 17$) invariably shows two trivalent associations at meiosis; (3) the preservation of the intermediate evolutionary steps, *intraspecifically*, in several species of two closely allied sections of *Podocarpus* where the number of chromosomes ranges from $2n = 38$–33 (of the 8 species examined so far, 6 contain presumed fusion heterozygotes, to the number of 14 in a total of 21 immature plants); (4) the comparison between the equivalent chromosome arms of homozygotes and heterozygotes of lower chromosome number respectively, which favours the concept of a *descending* basic series.

At present it would be unwise to insist upon the one-way scheme proposed; nevertheless such a sequence would represent continuous progress toward an economy of centromeres and stability of chromosomes.

The present instance of chromosomal polymorphism, which embraces not only the homozygous (or partly "saturated") but also the heterozygous (or transitional) phases of the evolutionary succession, appears to be the most striking example as yet described in the plant kingdom and is a surprising discovery amongst long-lived woody plants generally.

These findings are briefly discussed in relation to taxonomic questions.

HAMERTON, JOHN L., and CHARLES E. FORD. Chromosome Polymorphism in Wild Populations of the Common Shrew, *Sorex araneus*. *M.R.C., Radiobiological Research Unit, Atomic Energy Research Establishment, Harwell, Didcot, Berks., and British Museum (Natural History), South Kensington, London, England.*

Sorex araneus is a successful and widely distributed palearctic insectivore. It has recently been established that the species has 2 unusual features of cytogenetic interest: (*a*) males have 3 sex chromosomes (XY_1Y_2) whereas the females have the normal 2 (XX); (*b*) numerical chromosomal polymorphism of the Robertsonian (fragmentation-fusion) type occurs. The latter is complex: 6 medium sized autosomal elements (3 pairs) may occur in alternative metacentric or twin acrocentric states. The actual diploid number therefore varies from 21 to 27 in different males and from 20 to 26 in different females. Of the 27 theoretically possible autosomal combinations 22 have been identified.

Most of the animals examined were obtained from 3 localities within the county of Berkshire. The results show that significant differences in the frequencies of the alternative chromosomal states may occur from year to year in the same locality, and from locality to locality in the same year.

In 1957 small samples were obtained from 3 other more widely separated localities in Britain. Surprisingly all but one of the 19 animals were complete structural homozygotes. However, there was a different type at each locality. In all, 8 different structurally homozygous types are possible.

The case is presumably an example of balanced rather than transient polymorphism, but the evidence gathered so far does not demonstrate this decisively. It does, however, suggest that shrew populations are commonly small and relatively isolated from one another. It will be the main task of the 1958 collecting season to obtain samples from still further localities to determine (*a*) under what conditions mixed populations occur, and (*b*) whether the distribution about the country of the homozygous populations is orderly or random. It is hoped to present preliminary results from studies to the Congress.

HAMILTON, KATHERINE F. *See* GRAHN, DOUGLAS.

HANNA, BERTRAM L. Measures of Group Divergence for Two and Three Allelic Systems. *Medical College of Virginia, Richmond, Va., U.S.A.*

The comparison of allelic frequencies in two groups of individuals may be accomplished using simple chi square tests if the frequencies of each independent

locus are considered separately. In estimating the genetic relationship among groups it is often desirable to combine information about the frequencies at numerous loci to obtain a single measure of group divergence. Hiernaux (Ann. Musée Royal du Congo Belge, 1956) has employed the summation of χ^2 values at different loci as a measure of divergence between human populations. This statistic is appropriate for use with loci having two alleles since $\Sigma\chi^2_{(1)}$ approximates the square of linear distance measured as unit normal deviates ($\chi_{\phi}{}^2{}_{(1)} \simeq k_{\phi/2}{}^2$). The variance of a $\Sigma\chi^2_{(1)}$ value estimated from r loci is given by

$$V = \sum_{i=1}^{r} \frac{p_i(2n_i - 6) + 1}{n_i p_i}$$

where n_i and p_i are respectively the number and the frequency of an allele at the ith locus. The significance of the divergence measured by an observed $\Sigma\chi^2_{(1)}$ is tested by determining its deviation from the value r expected under the null hypothesis. The inclusion of χ^2 values derived from loci having three alleles will invalidate this measure since $\chi_{\phi}{}^2{}_{(2)}$ approximates $-2 \log_e \phi$. $\chi^2{}_{(2)}$ values may, however, be transformed to normal deviates and summed together with those derived from two allelic systems. The significance of the divergence measured by this combined value may not be determined.

Values obtained with these measures of two and three allelic systems and those obtained by combining values are compared with the measures obtained by Hiernaux and by other workers.

HARLAN, JACK R. *Bothriochloa intermedia* A. Camus: A Study in Speciation. *Department of Agronomy, Oklahoma State University, Stillwater, Oklahoma, U.S.A.*

Forty-one accessions of *Bothriochloa intermedia* were studied critically for evidences of introgressive hybridization. On the basis of morphological variation patterns, it was concluded that sixteen of the lots had been derived from *B. intermedia* × *Dichanthium annulatum* hybridizations; four of the accessions showed evidence of being *B. intermedia* × *B. ischaemum* derivatives; four lots showed strong evidence for introgression with *B. pertusa*; three lots apparently had characters derived from *Capillipedium parviflorum*, and the residual fourteen accessions of *B. intermedia* constituted a hodge-podge of material of diverse origin which, for the moment, defies analysis. Some experimental evidence is available to support these conclusions. The assumed *B. intermedia* × *Dichanthium annulatum* derivatives have been called the gangetica type of *B. intermedia*. All accessions of the gangetica type so far tested are facultative apomicts and hybrids have been made artificially between gangetica forms and several types of *B. ischaemum, B. intermedia, B. pertusa, Dichanthium annulatum* and *Capillipedium parviflorum*. One accession of *B. intermedia* × *B. ischaemum* was also tested and found to be partly sexual. Accessions of *B. ischaemum, Capillipedium parviflorum,* and *B. pertusa* so far tested have all been highly apomictic as well as most lots of *B. intermedia* other than gangetica types. Other species such as *B. ewartiana* have probably also contributed germ plasm to this remarkable synthetic taxon. *Bothriochloa intermedia* appears to be a repository for germ plasm from at least five species belonging to three genera.

HARRINGTON, JAMES B. FAO Regional Plant Breeding Projects. *Food and Agriculture Organization of the U.N. Rome, Italy.*

During the past eleven years the Food and Agriculture Organization has been conducting three regional projects in plant breeding: the Rice Improvement Project under the International Rice Commission; the Hybrid Maize Project in Europe and the Mediterranean region; and the Wheat and Barley Breeding Project in the Near East. These projects, through regional meetings of technical personnel, regional training centres, the services of regional consultants, the awarding of fellowships, and the exchange and cooperative testing of breeding material, have contributed to a notable increase in (1) the number of well-trained technical personnel, (2) the number of cereal breeding laboratories, greenhouses, nurseries and field tests, (3) the understanding of the economic value of plant breeding research and its dependence on genetics and statistics, and (4) the development of superior new varieties.

The Maize Project (11 years old) has promoted the replacement of ordinary maize by hybrid maize and the adoption of the specialized procedures for breeding, producing and utilizing hybrid maize. The project has already resulted in increased maize production estimated at $68,000,000 annually. The Rice Project (8 years old) has promoted genetic and physiologic studies and featured extensive crossing of *indicas* with *japonicas* in connection with breeding for high response to chemical fertilizers. A number of newly developed rice varieties are ready for distribution. The Wheat and Barley Project (6 years old) has aroused new interest in the improvement of these ancient crops. Special features are the cooperative nurseries of rust and smut resistant genetic stocks from all parts of the world and the regional rust race survey. Promising new varieties have been developed and are being tested.

The cooperation of the countries participating in these regional projects is excellent. The three projects illustrate how a United Nations' technical agency can help on a regional basis to increase food production where it is most needed.

HARRIS, H., and E. B. ROBSON. The Genetics of Hypophosphatasia: The Excretion of Ethanolamine Phosphate in Clinically Normal Relatives. *London Hospital Medical College, Galton Laboratory, University College, London, England.*

Hypophosphatasia is a rare osteodystrophic disorder. One of its most striking biochemical features is a greatly increased excretion of ethanolamine phosphate in the urine.

The families of 14 individuals presenting with this condition have been investigated. No instance of parental consanguinity was observed. Out of 13 sibs of the propositi 2 were diagnosed as having hypophosphatasia. No cases of the disease were found among other close relatives. Urine specimens from 360 members of the families and from 100 normal controls were examined by paper chromatography following electrolytic desalting. The quantity of urine used in each case was standardised against the creatinine content of the urine. The only abnormality noticed was a slight but definitely increased excretion of ethanolamine phosphate in the urine of some of the relatives. It is suggested that the clinically affected individuals are homozygous for a rare gene which in heterozygotes leads only to a minor biochemical abnormality. The frequency of individuals in the

different relationships who show this increased excretion of ethanolamine phosphate is consistent with the hypothesis that such individuals are heterozygotes, but that only 60% of presumed heterozygotes can be detected by the chromatographic methods used. Neither sex nor age appears to affect the excretion of ethanolamine phosphate.

The severity of the disease and the age at which the osteo-dystrophy becomes apparent are very variable. In the present material and in published cases sibs resemble each other rather closely in these respects. This suggests that more than one gene may be involved. Further heterogeneity is revealed by the fact that different sibships have different rates at which the presumed heterozygotes are manifest. Taking those sibships which contain the parent of a clinically affected case and classified according to the level of ethanolamine phosphate excretion in that individual, then among 51 sibs of parents whose heterozygous nature is manifest, 18 also excreted ethanolamine phosphate, but amongst the 25 sibs of parents who were not manifest heterozygotes only one excreted ethanolamine phosphate.

HARRISON, G. AINSWORTH. High Temperature Responses of Inbred and Hybrid Mice. *University of Liverpool, Liverpool, England.*

Harrison (J. Physiol. *138*: 54–55) has demonstrated that both the morphological and physiological changes that occur when mice are reared at high temperatures facilitate survival at a high lethal temperature. The comparative adaptability of inbred and F_1 hybrid mice to high temperatures has, therefore, been investigated. Animals were reared from three to eight weeks of age at either 70° F.D.B. 61° F.W.B. (control reared) or 90° F.D.B. 85° E.W.B. (heat reared) using a split litter experimental design and then exposed to 107° F.D.B. 85° F.W.B. It has been shown that (1) both control and heat reared C57BL x RIII hybrids survive longer than correspondingly treated C578L and RIII inbreds but less well than A inbreds; (2) the difference in the survival time of the control and heat reared hybrids is significantly greater than the difference between control and heat reared RIIIs. But the capacity of the C57s and As to acclimatize to the heat is the same as that of the hybrids.

Although there is evidence from an analysis of growth rates and variances of body weight that the magnitude of the response of the As is accentuated by their inability to adapt to the control conditions as well as they adapt to the heat, by every criterion studied the C57s appear to be as developmentally flexible as the hybrids.

The results therefore support the hypothesis that it is not heterozygosity as such which determines fitness but the greater likelihood of obtaining, in a naturally outbreeding organism, an adaptive genotype in heterozygotes than in homozygotes.

(This work was mainly undertaken in the Department of Anatomy, University of Oxford.)

HARTE, CORNELIA. Physiological Genetics of Leaf Development. *Institut für Entwicklungsphysiologie, Köln, Germany.*

The connections between genetic structure and physiological reactions are very well known, whereas the phenogenetics of morphological characters of higher

plants are not often the object of research. We, therefore, took mutants which change the leaf form of *Antirrhinum, Oenothera* and various trees, and tried, by comparing them with the normal forms, to discover when and how the different genes act in the development of the leaves. We examined the morphological differentiation of the young leaves in the bud, and the histologic structure of leaves in different stages of development on microtome slides. Furthermore, we took measurements of growing leaves and of different characters of the full-grown leaves (length, width, and size of cells in different tissues, hairs and stomata in the epidermis). Examination of several mutants of different plants revealed the common trends of gene action. The development of the leaves comprises several distinct steps. In going from one step to the next, new meristems are formed and in the small-leaf mutants the beginning of the growth in a new direction is delayed. The development then proceeds too long in the one direction. The growth of the meristems is terminated by the onset of differentiation in the cells without regard to the actual growth of the leaf. In consequence the leaves of most mutants have some similarity to the forms exhibited by normal leaves in different stages of their development. Not only is the form of the leaves influenced, but also the size of the cells and the differentiation of some tissues. We cannot say that any of these genes act on different steps of leaf formation. In every leaf there are correlations between different steps of development, and we find regulating mechanisms when the development is disturbed genetically. We find what is called "relational-pleiotropy" by Hadorn.

HARTMAN, P. E., Z. HARTMAN, D. SERMAN, and J. C. LOPER. Genetic Complementarity in Histidineless *Salmonella typhimurium*. *Department of Biology, The Johns Hopkins University, Baltimore, Maryland, U.S.A.*

Linkage tests by complete transduction place the sites of mutation of over 140 independently isolated histidineless derivatives of *Salmonella typhimurium* within a small chromosome region. About 80% of the sites are separable by recombination. Of the 10% mutants not giving recombinants in reciprocal crosses, most may be differentiated from their partners by a characteristic level of negative interference, specific for each mutant. In approximately 10% of the mutations each mutation occupies many mutational sites, most frequently covering one or several *complete* loci. Most multisite mutants are exceptionally stable, but a few revert to prototrophy with high frequency.

The linearly arranged mutational sites have been divided into eight groups (loci H-E-F-A-B-C-G-D) on the basis of genetic mapping by complete transduction, biochemical criteria, and over 6,500 abortive transduction combinations for genetic complementarity. The loci are restricted to sharply defined and non-overlapping sub-sections of the histidine region; some appear polarized, leaky mutations occurring terminally. Six loci are primarily concerned with specific, individual enzyme reactions in L-histidine synthesis; the gene order reflects the order of the biochemical steps. The H and E loci may have more complex functions in enzyme control.

*Inter*locus tests show that genetic complementarity is generally demonstrable but is heterogeneously expressed, depending upon certain very specific mutant combinations. Particular mutations may secondarily affect reactions controlled by certain nearby loci; the presence of isoalleles is not excluded in other cases. In *intra*locus tests, negative responses have always obtained among mutants, respectively, of loci

H, E, F, A, C and G. Aberrant *intra*locus complementarity occurs with high frequency in locus B (imidazoleglycerol dehydrase), indicating four subunits. Rare exceptional positives have been noted in locus D (L-histidinol dehydrogenase), especially in combinations involving *hi-10* and *-208,* located at opposite ends of the locus.

HARTMAN, Z. *See* HARTMAN, P. E.

HARVALD, BENT, and MOGENS HAUGE. A Catamnestic Investigation of Danish Twins. *University Institute for Human Genetics, Copenhagen, Denmark.*

It is the purpose of our study to elucidate the role played by heredity in the aetiology of various diseases through a catamnestic investigation of an unselected twin series, comprising all twins born in Denmark in the years 1870–1910. Until now the data of more than 11,000 pairs have been procured. However, in only about 3,500 of these pairs have both twins reached the age of 5 years, and only these pairs have been included in the present study. For all these pairs information has been procured of disabilities of all sorts. In all cases of hospitalization, the case records have been studied, and all death certificates, autopsy records, histological examinations, etc., have been scrutinized. In this way it has been possible in most cases to establish a reliable diagnosis.

For most diseases manifesting themselves at older ages the importance of hereditary factors seems to be rather small. Therefore, nearly the same rate of concordance was found in monozygous and dizygous twin pairs for different forms of malignant growth and for different forms of arteriosclerotic disease. On the other hand a significantly higher concordance in monozygous than in dizygous twin pairs was found in bronchial asthma, diabetes, gall stones, toxic diffuse goitre and atoxic goitre, migraine, schizophrenia, manic-depressive insanity and mental deficiency.

Special attention has been paid to several other questions such as longevity, fertility, frequency of twin births in the families of twins, criminality and others, but the results are not yet available for discussion.

HASIMOTO, HARUO. Deviation of Sex Ratio Caused by Temperature Treatment of Silkworm Eggs Immediately after Deposition. *Matsumoto Branch, Sericultural Experiment Station, M.A.F., Matsumoto, Japan.*

In the silkworm the maturation division of the egg nucleus and fertilization occur immediately after the deposition. The author successfully induced dispermic androgenesis in the silkworm in 1934.

In the experiment described here eggs were transferred immediately after being laid by mated female from room temperature to a refrigerator at 2.5°C, and after 10 days of cold storage were subjected to a temperature at 40–41°C for 60 minutes. The materials used in the present study were the eggs laid by *yellow-bloody, sex-limited zebra* females mated with *lemon* or *sex-linked translucent* males. In both matings the progeny were exclusively male. Almost all the males

showed the recessive character of the male parent. This is due to androgenesis. In addition, it is highly probable that the sperm nuclei in the egg can resist the cold, while the egg nucleus is not capable of surviving the same treatment.

In the experiment three lots of eggs were transferred immediately after deposition to a refrigerator at 2.5°C, O°C, and —2.5°C, respectively. After 43 hours of cold storage they were subjected to 39°C for 60 minutes. The sex ratio in the 0°C and —2.5°C lots showed a remarkable excess in the number of females, while in the 2.5°C lot it was nearly 100:100. The following generation was tested with the result that the majority of the females in the lots which showed an abnormal sex ratio were triploid or tetraploid, while those in the lot in which the sex ratio was normal were diploid. It seems clear that the cold treatment caused polyploidy in the female pronucleus.

Therefore, a difference in the temperature treatment of eggs induces a wide range of deviation in the sex ratio of the resulting silkworms.

HATTERSLY-SMITH, M. *See* ARMSTRONG, J. M.

HAUGE, MOGENS. *See* HARVALD, BENT.

HAYASHI, NORIO. *See* FURUHATA, TANEMOTO.

HEBB, CAROLINE RAUT, and THOMAS P. SINGER. The Adaptive Formation of Succinic Dehydrogenase during Aeration of Anaerobically Grown Yeast. *Detroit Institute of Cancer Research and Wayne State University College of Medicine, Detroit, Mich., U.S.A.*

The adaptive synthesis of succinic dehydrogenase induced by oxygen has been studied in normal yeast in an attempt to learn more as to the nature of such gene-controlled enzymes which require an external inducer for their production. Red Star yeast was grown anaerobically and then aerated in buffer and glucose to induce the formation of various oxidative enzymes in the absence of growth. At intervals during the oxygen adaptation, samples of yeast were broken in a Nossal shaker, and the subcellular particles sedimenting at 81,000 x g in 15 minutes were assayed for succinic dehydrogenase activity (1) using phenazine methosulfate as electron acceptor to assay the maximum activity of the primary dehydrogenase and (2) using cytochrome-c as acceptor to measure the succinate-cytochrome-c interaction which involves participation of an electron transport chain between the succinate and cytochrome-c. In a typical experiment, the activity of the primary dehydrogenase increased some 5-fold from 0.046 μM succinate/min./mg. protein at 30°C (15% of the activity of aerobic yeast) at the beginning of aeration to 0.25 μM succinate/min./mg. protein (85% of aerobic activity) after 23 hours aeration. At the same time the activity of the entire complex increased as much as 90-fold, starting from an extremely low activity of 0.0013μM succinate/min./mg. protein or less (around 1% of the aerobic activity).

A reduction of succinic dehydrogenase activity in anaerobically grown yeast of 96%–97% of that of aerobic yeast has been reported (Slonimski, La Formation des enzymes chez la levure, Paris, 1953, pp. 135–136) when measured using methylene blue or cytochrome-c as electron acceptors. However, comparison of the assay using cytochrome-c with that using phenazine shows that while succinic dehydrogenase in anaerobic yeast is decreased several fold compared with that in aerobic yeast, the components of the electron transport chain between the primary dehydrogenase and cytochrome-c as well as the oxidations involving these are essentially absent. Therefore the mechanism of enzymatic adaptation consists primarily of the formation of the electron transport chain.

HECHT, ADOLPH. Partial Restoration of Fertility in a Sterile *Oenothera* Mutant following Infection by a Parasitic Moth Larva. *State College of Washington, Pullman, Wash., U.S.A.*

A mutant of *Oenothera parodiana* was first observed in cultures of this South American species grown in Chicago in 1946. Mutant plants are characterized by a monstrous type of floral development, such that four bracts are formed at the juncture of the hypanthium with the ovary; the hypanthium is much reduced in length and the floral organs within the calyx are distorted in shape and develop only incompletely. No pollen is formed and the bud ordinarily fails to open, often prematurely aborting and drying. The meiotic configuration of the non-mutant plants is a circle of ten chromosomes and two pairs. Progeny obtained by selfing any of them gives rise to some plants with monstrous flowers. Over a seven-year period 773 of 3,506 plants, or 22%, were phenotypically mutant. It appears that the normal plants must all be heterozygous, and that certain expected classes of progeny die at some stage prior to seed germination. Support for this interpretation comes from one instance of obtaining approximately 50% plants with monstrous flowers by a successful pollination of a monstrous-flowered plant, and from two instances of 100% plants with monstrous flowers following X-irradiation of the dry seeds. One circumstance favoring the occasional development of a few partially fertile flowers on an otherwise monstrous-flowered plant appears to be the infection of the ovaries by an insect larva. Infected ovaries have a hole in them where the larva presumably entered, and these ovaries are more strongly angled than those not infected. Adults of the insect were obtained and have been identified as *Mompha circumscriptella* of the microlepidopteran family Momphidae. It remains to be determined whether development is stimulated by some secretion from the larva or merely as a consequence of the wounding and mechanical irritation.

HERSKOWITZ, IRWIN HERMAN. Genetic Recombination Induced by X-rays in Female Germ Cells of *Drosophila*. *Biology Department, Saint Louis University, St. Louis, Missouri, U.S.A.*

The kinds and frequencies of X-ray induced genetic recombination in female germ cells of *Drosophila* depend upon their stage in differentiation. In most mature cells many breaks are produced. Some rejoining occurs soon after certain treatments, but is delayed after higher doses are delivered more quickly. When the

two ends undergoing an exchange (non-restituting) union are from non-homologous chromosomes, the result is "half-translocation"; when they are from the same tetrad, the result is deletion or inversion if one strand is broken twice, and is either duplication or deficiency or both if two strands are involved. Here the breaks are presumably sometimes at nearby, but not necessarily identical, loci producing a "pseudocrossover." Many breaks fail to rejoin, at least when there is more than one per cell. Thus, the union producing a half-translocation usually is not accompanied by the reciprocal joining of the other two broken ends.

In viable exchanges the breaks often occur in heterochromatic regions because these, as compared with euchromatic ones, are either more breakable, less restitutable, in closer proximity, more viable in aneuploid condition, or any combination of these factors. While such heterochromatic recombinations probably produce relatively few recessive lethals they frequently cause dominant lethality via "non-disjunction" or the retention in the egg after reduction of an unjoined broken end, or a dicentric chromosome, or a eucentric one grossly aneuploid.

When progressively immature germ cells are irradiated half-translocations and dominant lethals decrease in frequency, X-ray induced "crossovers," presumed to occur between exactly homologous loci, increase while pseudocrossover frequency apparently is unchanged. Dominant lethals, half-translocations, pseudocrossovers and X-ray induced crossovers increase when the X-ray dose is delivered either more intensely, or to females previously dehydrated. Accordingly, all are attributed in large measure to multiple independent X-ray hits, many dominant lethals and all half-translocations and pseudocrossovers resulting from multiple independently produced breaks.

HETZER, H. O., J. H. ZELLER, and R. L. HINER. Three Generations of Selection for High and Low Fatness in Duroc Swine. *U.S. Department of Agriculture, Agricultural Research Center, Beltsville, Md., U.S.A.*

Results of selecting for high and low fatness in two lines of Duroc swine through 3 generations are presented. Starting in 1954 with a group of 82 pigs from 16 litters, foundation animals for the 2 lines were selected largely on an intra-litter basis after foundation animals for an unselected control line had been randomly selected from the same litters. The primary criterion of selection was individual backfat thickness at a live weight of 175 pounds and, except for a few late farrowed pigs in the 3rd generation, all normal pigs weaned in each line each generation were probed. Usually 6 boars and 12 gilts were selected for parents of each generation in the selected lines and 12 boars and 12 gilts in the control line, care being taken to keep the relationship between mates at a minimum. The selection actually practised on the basis of backfat thickness resulted in mean differences between the high and low lines of .04, .19 and .37 inches, in the first, second and third generations, respectively. Starting with an average of 1.49 inches, the average increased to about 1.70 inches in the high line and decreased to about 1.33 in the low line. The largely intra-litter selection in the foundation stock was about .19 effective compared with estimates of .58 and .62 from the response observed in the second and third generations. The latter two values agree rather well with the heritability estimate of .59 from regression of progeny on mean of parents within lines and generations, indicating that backfat thickness is rather highly

heritable. However, realized heritabilities computed according to Falconer differed somewhat between the high and low lines, .57 versus .41 suggesting that selection was relatively less effective for low backfat than for high. Carcass data obtained on samples of pigs showed the low line exceeding the high line in percentage yield of lean cuts and, at the same time, averaging lower in yield of fat cuts.

HEXTER, WILLIAM M. Probable Gene Conversion in *Drosophila*. *Amherst College, Biology Department, Amherst, Mass., U.S.A.*

Attached-X analysis of *garnet* in *Drosophila* yielded data not easily explicable by orthodox crossing-over. Four "different alleles" (g^2, g^3, g^4 and g^{53d}) were analyzed in three independent tests. In each test one arm of the attached-X contained $g^{53d}sd$, the other wy^2 and one of g^2, g^3 or g^4. The latter three alleles, indistinguishable from each other, give "brown" eye while g^{53d} gives "orange." Further phenotypic separation can be made by combination with w^a. Genetic tests yielded wild type females in the following proportions which are statistically similar: g^2-g^{53d}–4/78,843; g^3-g^{53d}–5/95,139; g^4-g^{53d}–3/50,031. Based upon the observed recombination of the markers preliminary analysis results in an interpretation of pseudoalleles with g^{53d} to the left of g^2, g^3 and g^4, the latter three presumably at the same locus. Such an interpretation demands recovery of the presumed double mutant, g^{53d} g^2, ^{3} or ^{4}. Phenotypic evidence indicated no double mutant. Attempts to recover g^{53d} from such a presumed combination yielded: $g^{53d}g^2$–0/90,104; $g^{53d}g^3$–0/153,994; $g^{53d}g^4$–0/115,305. The probability of 0/359,403 due to chance is less than 0.001. It is argued therefore that at least three wild-type females arose by conversion of g^{53d} to g^+ accompanied by crossing-over between wy^2 and *sd*. Upon test another wild-type female proved to have $wy^2g^{\text{"brown"}}$ in one arm and *sd* in the other. This can be explained by conversion of g^{53d} to g^+ unaccompanied by crossing-over or, less likely, by a double crossover within a short distance. A final female phenotypically *sd* with wild-type eye and proven heterozygous for g^{53d} can be explained by conversion of g^{53d} to g^+ accompanied by crossing-over between *sd* and the kinetochore or, less likely, by a triple crossover within a short distance. It is not proven that the garnet locus is, in fact, pseudoallelic. (This work has been supported by research grant RG–C–4599 from the National Institutes of Health.)

HIGHKIN, HARRY R. Transmission of Phenotypic Variability in a Pure Line. *California Institute of Technology, Pasadena, Calif., U.S.A.*

During the course of physiological and genetic studies of heat resistance in peas (*Pisum sativum*), conducted in the Earhart Plant Research Laboratory, it was found that the F_1 population of a cross between two pure lines exhibited a variance in growth which exceeded considerably that of either parent. Analysis of the data showed that the excessive variance could be attributed entirely to the differences among the environments of the different pollen sources. Results of this and other experiments pertaining to dauermodification type inheritance will be discussed.

HILL, BERTON F. *See* WOLFF, GEORGE L.

HINER, R. L. *See* HETZER, H. O., *et al.*

HINTON, CLAUDE W. Cytological Demonstration of Cleavage Anaphase Bridges in *Drosophila melanogaster*. *Department of Zoology, University of Georgia, Athens, Ga., U.S.A.*

The behavior of an unstable ring-X chromosome called w^{vc} of *Drosophila melanogaster* has been explained by anaphase bridge formation during cleavage mitoses followed by breakage of the bridge to cause dominant lethality or by loss of the bridge to produce either gynandromorphs or X0 males. An attempt to confirm this model consisted of cytological examination of Feulgen whole mount preparations of third through eighth cleavage eggs derived from rod-X females crossed either to w^{vc} males or to males carrying the stable X^{c2} ring chromosome. The frequency of late anaphase and telophase figures with bridges was 1.94% (N = 2,516) and 0.28% (N = 1,084) for the two groups, respectively; these frequencies are not corrected for the expectation that approximately one-half of the embryos carry the Y chromosome rather than a ring-X. Studies of other mitotic abnormalities found in this material are in progress. (Work supported by U.S. Public Health Service Research Grant C-3000.)

HIROTA, YUKINORI. The Effect of Acridine Dyes on Mating Type in *Escherichia coli*. *Department of Genetics, University of Wisconsin, Madison, Wis., U.S.A.*

The mating types of *Escherichia coli* are determined by the presence of a transmissible agent, F. The study reported here reveals that it is possible to obtain F— cells at will from F+ with high efficiency (90–100%) growth in broth containing acridine dyes (acriflavine and acridine orange). (1) the F— strains obtained are genetically fixed in this mating type, also the other F— reported by other workers. (2) No effect has been observed yet on characters which show chromosomal segregation or on the production of colicine, E. (3) F— cells appear in F+ populations in one-half to one hour after exposure to acridine dyes, and then the rate increases rapidly. (4) Some strains of intermediate compatibility were obtained. (5) The stable Hfr_1 strain (High frequency of recombination), W1895, is not affected by the treatment. The efficiency is influenced by the medium and by the temperature. The killing action and F removing action of this environmental condition are not correlated.

A simple working hypothesis to interpret these phenomena is that F is inherited as a plasmid.

HIRSCHORN, KURT, and CHAS. F. WILKINSON, Jr. The Mode of Inheritance in Essential Familial Hypercholesterolemia. *Department of Medicine, New York University Post-Graduate Medical School, New York, N.Y., U.S.A.*

During the past twenty years, a number of families carrying the trait of essential familial hypercholesterolemia with xanthomatosis have been studied by various investigators to determine the method of inheritance.

Almost all of these studies have accepted and confirmed the concept of the inheritance of hypercholesterolemia as an autosomic dominant. There remain, however, two schools of thought: one considering it to be a complete dominant, and the other calling it an incomplete dominant, with homozygosity a prerequisite for xanthomatosis, increased incidence of coronary heart disease, and very high cholesterol values.

We wish to report several new sibships with this defect. Once again, the dominance of hypercholesterolemia is borne out. These sibships seem to show the incomplete mode of dominance in that all very high cholesterol values and xanthomatosis are found only in homozygous individuals.

It is our opinion that these two methods of inheritance are not incompatible with each other. We may assume that only one gene locus is involved since the offspring in all studies show a monozygotic ratio. There exist many samples in experimental genetics of multiple alleles of one factor, showing different degrees of dominance, and even occasionally mutating to recessive form. After analysis of our material, as well as re-analyzing all previously reported families, we strongly believe that such multiple alleles may be responsibe for the various types of inheritance reported in essential hypercholesterolemia.

HOCHMAN, BENJAMIN. Wild-Type Isoalleles in Natural and Laboratory Populations of *Drosophila*. *Department of Genetics, University of Utah, Salt Lake City, Utah, U.S.A.*

An examination of the fourth chromosomes of 36 laboratory and 4 natural populations of *Drosophila melanogaster* was made in order to estimate the frequency of wild-type isoalleles at the *cubitus interruptus* (*ci*) locus. The method employed permits the classification of ci^+ alleles into two categories: one group (class A) is always dominant over the mutant allele *ci*; the other (class B) comprises those ci^+ alleles which allow the expression of the mutant phenotype (interruptions in wing vein four) in some the heterozygous ci^+/ci flies. Class B alleles may be seriated further by penetrance and expressivity studies.

All of the alleles from natural populations were in class A, while in two of the laboratory stocks a class B ci^+ gene was uncovered. In homozygous condition each allele conferred the normal phenotype. The two domesticated stocks in question are of widely separated geographical origins.

This low incidence of class B ci^+ alleles is somewhat in conflict with earlier findings of Stern and Schaeffer (1943). They examined three laboratory stocks and found each to contain a measurably distinct class B allele. Two of the three genes found by Stern and Schaeffer gave very low percentages of flies with interrupted veins in heterozygous combination with the *ci* allele. Such class B alleles with very low "penetrance" may have escaped detection owing to the less sensitive testing technique utilized in the present investigation.

The apparent absence of class B ci^+ isoalleles in natural populations (over 80 trapped males have been tested) is not surprising considering the results obtained in population cage experiments with ci^+ isoalleles. If the adaptive values ascertained in the artificial populations studied are comparable with those existing in nature, class B ci^+ isoalleles should be rare.

HOGE, A. R. *See* SCHREIBER, GIORGIO, *et al.*

HOLLANDER, W. F. Sperm Abnormality of a Mutant Type Involving the *p* Locus in the Mouse. *Department of Genetics, Iowa State College, Ames, Iowa, U.S.A.*

A mutant pink-eyed dilute obtained from X-irradiated sperm (500r) has been analysed. Though identical with *p* in color effects, it is abnormal in other ways: homozygotes are about a third lower in body weight than comparable heterozygotes, and show a slight choreic behavior. When weaned they have great difficulty in chewing food biscuits through ¼-inch mesh wire. Adults often show abnormal wearing of the upper incisors. All homozygous males tested (about 50) have been sterile, except one which was partially fertile for a brief time. Sterility seems in part due to failure to copulate, but in addition most of the sperms produced are malformed, lacking the hook. Motility of the sperm is normal in spite of head deformities. Homozygous females have usually been able to produce one or a few litters of viable young, but milk production is poor and the young are rarely raised. The mutant acts as a simple recessive both to *p* and the normal allele. The pleiotropic effects suggest that the mutant may be a deficiency. If so, not a very large segment would be involved, since it is not lethal, and crossing over with the *c* locus is apparently not reduced. The symbol p^s is employed. (This study was assisted by Contract No. AT(11-1)107 from the Atomic Energy Commission.)

HOLLOWAY, B. W. Genetic Recombination in *Pseudomonas aeruginosa*. *Department of Bacteriology, University of Melbourne, Carlton, Australia.*

The occurrence of genetic recombination in *Pseudomonas aeruginosa* has been demonstrated. Recombination of both selected and non-selected characters has been demonstrated and it is apparent that some system of ordered genes is operating. A compatibility system dependent on a factor FP occurs such that mating takes place between FP+ and FP— strains. FP— strains may be converted to FP+ by contact with FP+ strains. The nature of the inheritance of the FP factor and its influence on the segregation of genetic markers has been investigated. One of the interfertile strains is susceptible to a pyocin liberated by another interfertile strain and the general characteristics and inheritance of this agent have been studied in relation to other inherited characteristics of the strains. The general characteristics of the genetic mechanisms studied in these strains point to its similarity to those occurring in *Escherichia coli*.

HOLT, SARAH B. The Inheritance of Ridge-Count in Finger-Prints. *Galton Laboratory, University College, London, England.*

The method of ridge-counting, which gives a measure of pattern size, has been used for genetic studies on the dermal ridge patterns of fingers. It has been shown by statistical analysis of data from parents and children, sibs and twins, that the total ridge-count (i.e., the sum of the counts on the ten fingers of an individual) is

inherited. The genes concerned are additive with independent effect, but without dominance. There is evidence also that the variability of ridge-counts from finger to finger has a genetic basis.

The demonstration includes frequency distributions for total ridge-count for males and females in a British population sample and diagrams to illustrate the correlations for total ridge-count between (*a*) parents and children, (*b*) sibs, (*c*) monozygotic twins, and (*d*) dizygotic twins.

HOUGH, L. FREDRIC, and GERALD M. WEAVER. Irradiation as an Aid in Fruit Variety Improvement. *Department of Horticulture, Rutgers University, New Brunswick, N.J., U.S.A.*

Peach trees grown one and two years in the Brookhaven Gamma Field have produced mutations in the season of ripening, firmness and texture of the fruit flesh, and the freestone condition of the pit. A late-ripening mutant of Elberta holds promise of becoming a commercial variety.

In vitro and *in vivo* germination has been observed and seedlings have been obtained from dry sweet cherry pollen subjected to gamma radiation at dosages of 100,000 to 400,000 roentgens. Relatively low dosages of ionizing radiations have been observed to have a stimulating effect in two instances. A stimulation in fruit set of sweet cherries was observed using dry pollen which had been treated with 25,000 and 50,000 roentgens acute gamma irradiation. The increased fruit set was apparently due to induced parthenocarpy as the seed set was very low. Thermal neutron dosages of 2 hours' duration at a 7.52×10^6 neutrons/cm.^{2}/sec. flux have been found to greatly stimulate initial seedling vigor so that a greater number of seedlings can be transplanted. This effect has only been observed with seedlings from weak seeds. This promises to be of practical value in breeding for very early-ripening peaches.

Current research to determine the most efficient method of irradiating fruit species includes chronic gamma irradiation, semi-chronic gamma irradiation during a particular stage of plant growth, and acute irradiation of buds, pollen, and seeds. Fruit seeds show the greatest response to irradiation when they are irradiated just as they are begining to germinate.

HOUGH, L. FREDRIC. *See* BAILEY, CATHERINE H.

HOVANITZ, WILLIAM. The Helical Structure of Giant Chromosomes. *Department of Biology, California Institute of Technology, Pasadena, Calif., U.S.A.*

Isolated chromosomes have indicated a compound helical structure with many orders of helices ranging in size from molecular coils of 50 Å in diameter through multiples of 100 Å, 200 Å, etc., up to microscopically visible coils such as are seen normally in microscopic cytological preparations. The unique position of the Dipteran giant chromosomes in presumably being constructed of transverse bands rather than helices seemed untenable in view of the universality of the helical structure of all other chromosomes. A revised outlook on this matter has indeed

shown that the "bands" of the giant chromosomes of *Drosophila* and *Chironomus* are turns of helices which have been visibly compounded from a hierarchy of smaller helices. The darkly staining portions of these chromosomes differ visibly from the lighter staining portions by the tighter coils of which they are composed. There is no need to postulate a different chemical structure of these parts, in fact at varying times of the growth of the organism, the one may become the other. The lighter staining portions are loosely coiled but show a continuity of coiled strands similar to that of the darker portions. The largest helices (bands) are apparently not a unit transversely, but are visibly formed by the meeting of innumerable smaller coils in close union. These coils can be relaxed from their tight "banded" appearance by the application of salt solution before fixation. Helices of various orders of size can be seen clearly in this condition.

HOWARD-FLANDERS, PAUL. The Effects of Nitric Oxide and Oxygen on Radiation-Induced Lethal Injuries in Bacteria and Yeast. *Donner Laboratory, University of California, Berkeley, Calif., U.S.A.*

The presence of oxygen during exposure is known to affect the production of a variety of radiation-induced injuries, including chromosome aberrations, dominant and recessive lethals and certain other mutations in a number of different organisms. Further insight into the mechanism of the oxygen effect may be obtained from the observation that, for all the micro-organisms so far tested, it has been found that nitric oxide enhances the effects of irradiation carried out under anoxia. In experiments on the bacillus *Shigella flexneri*, in which the criterion of survival was the ability to form colonies, it was found that the effects of oxygen and nitric oxide on the sensitivity to X-irradiation were similar in two respects. The same concentration of gas was needed to double the sensitivity, and the degree of enhancement of sensitivity at high gas concentrations was the same within experimental error. The similarities and contrasts in the actions of the two gases on radiation injury and on cellular metabolism will be discussed.

HRUBÝ, KAREL. Caterpillar Colour Inheritance in *Biston betularius* L. *Genetics Institute, Charles University, Prague, Czechoslovakia.*

The geometrid *Biston betularius* L. is polymorphic in the adult as well as caterpillar stage. Colour inheritance of the imago is well known. The caterpillars occur in different colour shades from pale greyish green to dark brown. They may be classified into two distinct categories: (i) without pigmentation, (ii) coloured. These categories are not merely modifications. The genotypical independence of caterpillar and imago pigmentation has been proved. (Dark and normal moths emerged from both coloured and colourless caterpillars in the same monohybrid back cross ratio 1:1.)

Crossings concerning only the caterpillar colour gave the following results. In the progeny of an initial cross 201 pigmented and 181 colourless caterpillars occurred (9:7, $\chi^2_{(1)} = 1.95$, $P = 0.16$). Two types of crossing were then performed: (i) pigmented $\times$ pigmented, (ii) colourless $\times$ colourless. In the progeny of the first cross there were 121 coloured and 40 colourless caterpillars (3:1, $\chi^2_{(1)} = 0.002$, $P = 0.92$). But the same ratio appeared in the progeny of the colourless

cross. There were 66 coloured (22 of them very dark) and 14 colourless (3:1, $\chi^2_{(1)} = 2.2$, $P = 0.15$, but greater agreement with the ratio 1:2:1, $\chi^2_{(2)} = 2.4$, $P = 0.30$). But monofactorial inheritance cannot be considered. The occurrence of pigmented, even strongly pigmented, caterpillars in the progeny of a cross colourless $\times$ colourless points to the polyfactorial complementary kryptomeric interaction.

There should be three pairs of complementary genes, viz., the basic factor for the production of the pigment *M* which is incompletely dominant, and two pairs of realisators *A* and *B*. The caterpillar is coloured only when all three pairs are represented in the genotype by the dominant alleles. The genes of these three pairs have moreover an additive effect, thus *MMAABB* producing very dark pigmentation and *MmAaBb* only a light brown colour. All results mentioned are in accordance with this supposition.

Hsia, David Yi-Yung, Shirley G. Driscoll, and Eva M. S. Gawronska. The Detection of Heterozygous Carriers in Hereditary Diseases. *Genetic Clinic, Children's Memorial Hospital, and Northwestern University Medical School, Chicago, Ill., U.S.A.*

The detection of heterozygous carriers of a hereditary disease is important for a variety of reasons: (1) it provides the immediate family with the information as to who are likely to have affected offspring. The prompt institution of treatment made possible by this foresight may bring about the successful therapy of such conditions as galactosemia or phenylketonuria; (2) it is useful as a means of determining the incidence of carriers for any given trait within the community and gives an indication as to whether the load of mutations is increasing or decreasing; (3) it is of great value in the understanding of pathogenesis of a given hereditary disease. The ability to detect the same biochemical defect in the heterozygote usually indicates that one is dealing with a primary and not secondary gene effect; and (4) the heterozygous carriers represent at least a potential medical hazard. Muller has estimated that each individual on the average carries eight mutant genes in heterozygous condition. Under normal conditions, these may only slightly affect his general performance in life, and perhaps increase his susceptibility to non-genetic diseases. In periods of stress, these could well represent the difference between extinction and survival.

The present paper will discuss some of the principles and newer biochemical methods which are proving to be useful in the detection of heterozygous carriers of hereditary traits in man. Detailed data obtained in our own laboratory on the following conditions will be presented: (1) phenylketonuria; (2) idiopathic hyperlipemia; (3) vitamin D resistant rickets; (4) cystic fibrosis of the pancreas; (5) glycogen storage disease (von Gierke's Disease); (6) galactosemia.

Hsu, T. C. Changes of Chromosomal Constitution and Host Compatibility of the Novikoff Rat Hepatoma Cells Grown *in vitro*. *University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Tex., U.S.A.*

The Novikoff rat hepatoma cells carried in Sprague Dawley rats contain two modes of chromosome number distribution, one at 39 and the other at near 78. The "diploid" cells (39 chromosomes) exhibit several new chromosomes to the normal

rat complement ($2n = 42$): a very large and two slightly smaller metacentric chromosomes and a small chromosome with a long satellite. The tetraploid cells show the doubling of these elements.

Three *in vitro* cell strains have been successfully maintained from the original tumor tissues. The chromosome pattern of the cells underwent a series of changes. First, there was a definite decline in the frequncy of the "diploids" and an increase in the "tetraploids"; then heteroploids arose. The chromosome number of the heteroploids ranged from far below to far above that of the "tetraploids." Besides, within each population, the combinations of individual chromosomes were as varied as the numbers. After 8–10 months of continuous cultivation, the populations became totally heteroploid with a rather narrow range of chromosome numbers. However, each strain has its own characteristic chromosomal distribution. At the present time, Strain N–1 is characterized by 67± chromosomes, N–3 by 60± chromosomes, and N–4 by 74± chromosomes.

Inoculation of the *in vitro* cells into young Sprague Dawley rats produced tumors in the early period of cultivation. During the stages of transformation a decrease in transplantability was noted. Cells from Strains N–1 and N–3, after reaching the present chromosome constitution, produce a small tumor but regress after two weeks. These rats would later reject the grafts of the *in vivo* Novikoff hepatoma. Cells from Strain N–4, however, still produce tumors but with marked decrease in malignancy.

(Supported in part by Grant #DRG-269C from Damon Runyon Memorial Fund for Cancer Research and Grant #P-133 from American Cancer Society.)

HUBBY, J. L. *See* GLASSMAN, E., *et al.*

HUESTIS, R. R., and RUTH S. ANDERSON. Genetic Change in an Axial Skeleton. *University of Oregon, Eugene, Ore., U.S.A.*

The modal presacral vertebral formula in Oregon *Peromyscus maniculatis* is C7T13L6. A relatively long spinous process is typically present on the second thoracic vertebra. This process may be lacking in some individuals, or in a smaller number may be located on the third thoracic vertebra. These three types of skeletons will be called P_2, P_0 and P_3 respectively. In a cage-bred sample of 332 individuals prepared to establish a norm, 80% of the skeletons were P_2; 13% were P_0; and 7% were P_3. By selection of P_0 individuals for parentage, which may be done by feeling between the vertebral borders of the scapulae, increases were obtained in the incidence of both P_0 and P_3 types and in the average number of ribs and presacral vertebrae. Selection of P_3 individuals for parentage by progeny tests continued this trend so that in a sample of 163 *P. maniculatis* obtained from P_3 parents, 7% of the skeletons are P_2; 26% are P_0; and 70% are P_3. The total modal number of presacral vertebrae in this selected group has become 27. In a comparison of young produced by other matings: $P_2 \times P_2$; $P_2 \times P_0$; $P_2 \times P_3$; $P_0 \times P_0$; and so on to $P_3 \times P_3$; the proportion of P_2 young diminishes; the proportion of P_3 young increases; and the proportion of P_0 young increases to a maximum in the $P_0 \times P_0$ mating and then diminishes.

Hughes, Roscoe D., and Harold W. Syrop. A Familial Study of the Agenesis of the Parotid Gland Duct. *Medical College of Virginia, Richmond, Va., U.S.A.*

Congenital absence of one or more salivary glands in man is an exceedingly rare anomaly even when this condition is a part of an associated generalized ectodermal dysplasia. Congenital absence or dysfunction of the parotid glands alone, or in association with dysfunction of the lacrimal glands, is still more uncommon. Only four cases of such anomalies have been reported in the literature and two of these were father and daughter. In the present study of a single family group there are nine affected individuals in three generations. Seven of these were available for examination. In each of these individuals there is an absence of the orifice of Stenson's duct and a marked diminution of saliva of the oral mucosa. The parotid gland is not palpable. This condition results in a characteristic which the members of this family term "dry mouth" and is associated with an abnormally high caries index. No other defects could be detected in the seven individuals except in two there is an absence or dysfunction of the lacrimal glands as well as the parotid glands. The distribution of the anomaly in this family group indicates that it can best be explained on the basis of a single autosomal dominant gene with a high degree of penetrance.

Hunter, A. W. S., and Marie Bragdo. The Cytology of Three Hybrids between Diploid and Hexaploid Plums (*Prunus* spp.). *Horticulture Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Canada,* and *Norges Landbrukhøgskole, Vollebekk, Norway.*

Meiosis was examined in the pollen mother cells of two tetraploid plums of the parentage St. Anthony ($2n = 16$) $\times$ Mount Royal ($2n = 48$) and in one pentaploid of the parentage Shiro ($2n = 16$) $\times$ Reine Claude ($2n = 48$). The tetraploids have a remarkably regular meiosis, 16 bivalents being formed in 73% of the cells at first metaphase in one and in 64% in the other. First anaphase separation results in 16 chromosomes at each pole in approximately 80% of the cells of both tetraploids. However, only 40% of the pollen stains with aceto-carmine and there is much variation in pollen grain size.

Meiosis in the pentaploid is much more irregular. Multivalents and univalents were found in all first metaphase cells examined, and anaphase separation of the chromosomes was usually unequal. Altogether 60% of the pollen stained with aceto-carmine, but again there was much variation in size.

All three hybrids bear moderate crops of fruit containing viable seeds under orchard conditions. In controlled crosses, no fruit resulted when the hybrids were used as male parents on diploid or hexaploid varieties. Viable seeds were produced by the three hybrids when they were used as female parents and pollinated by hexaploid but not by diploid varieties. These hybrids may be useful in the breeding of plums that combine the high fruit quality of hexaploids with the winter hardiness of some diploid species.

Hunziker, J. H. Karyotype Analysis in *Cupressus* and *Libocedrus*. *Instituto de Botánica, Ministerio de Agricultura, Buenos Aires, Argentina.*

Results: *Cupressus arizonica* = 22 (all V ?, 2 S, 2 sc ?); *C. funebris* = 22 V (2 S, 2 sc); *C. glabra* = 23 V (2 S, 2 sc), 1–2 nucleoli; *C. lusitanica* var. *lusitanica*

= 20 V (2 S, 2 sc) + 2 J; 20 V (2 s) + 2 J, 1–2 nucleoli; var. *Benthami* = 22 V (2 s), 1–2 nucleoli; 22 V (1 S, 1 s), 1–2 nucleoli; *C. macrocarpa* = 22 V (2 S); *C. sempervirens* = 22 V (8 S), 1–7 nucleoli; 22 (all V ?, 2 S, 2 sc ?); *C. torulosa* = 20 V (2 S) + 2 J; *Libocedrus chilensis* = 22 V (2 S, 4? sc), 1–2 nucleoli). In the preceding karyotype formulae the following abbreviations have been used:

$$\text{V} = \text{V-chromosome, brachial index} = \frac{\text{Length short arm}}{\text{Length long arm}} = 1\text{–}0.5;$$

J = J-chromosome, brachial index = < 0.5; S = linear satellite, distal segment longer than the proximal; s = round satellite, distal segment shorter than the proximal (in both S and s the satellites are separated from the rest of the chromosome arm by a long, nucleolar, thread-like, secondary constriction); sc = short secondary anucleolar constrictions (not always conspicuous).

Conclusions. The fact that two and four different karyotypes were observed in *C. sempervirens* and *C. lusitanica*, respectively, points to the existence of chromosomal variability within these species. In *C. funebris* and *C. torulosa* there are slight differences in the karyotype as described by Mehra and Khoshoo (J. Gen. *54*: 165–180) and as described here. In these two species there might also be intraspecific karyotype variation.

In the chromosomal race of *C. sempervirens*, having eight long secondary constrictions, all of them are presumably nucleolar because up to seven nucleoli were observed at resting stage. The supernumerary chromosome found in *C. glabra* is much smaller and appears to have a shorter centromere than the other chromosomes. Apparently in *Libocedrus chilensis* the long nucleolar secondary constriction is completely adjacent to the centromere. Further studies are being made in order to clarify this matter.

HUTCHINSON, A. H. The Principles Involved in Polygenic Selection from a Polyzygous Population of Douglas Fir. *Department of Biology and Botany, University of British Columbia, Vancouver, Canada.*

A genotypic evaluation of wind pollinated Douglas fir populations is premised on polygenic segregation involving various growth rates, frost sensitivity, and cyclic bud activity. In each case three or four allelic factors are required to satisfy the polyzygous states, which are evident from more than 80,000 readings. Simple equations in terms of N, the number of polygenic alleles, and n, the degree of parental heterozygosity, relate the phenotypes and genotypes of the progenitors to the class frequency distribution coefficients of the progenies $(1 + \text{I})^{n} \times 2^{2N-n}$. All population curves are reduced to the common base, 2^{2N}.

Each controlled biparental class frequency polygon of a specific polyzygous population is balanced and includes (n + 1) phenotypic classes from a possible (2N + 1) classes, each of which belongs to one of (factorial (N + 1)) effective genotypic classes. These genotypes may combine to produce factorial 2N + 1 class frequency series, as progenies. Wind pollinated, multiparental stocks are characterized by class frequency polygons which show predictable skewness, multiple peaks and extended plateaus.

Selection may be controlled genotypically on these bases. Examples of natural selection are presented.

Hyde, Beal B., and R. L. Paliwal. Sulfhydryls in Plant Chromosomes. *Department of Plant Sciences, University of Oklahoma, Norman, Okla., U.S.A.*

A number of SH reagents are known to affect both the physiology and, directly or indirectly, the structure of chromatin. We are studying the effect of N-ethylmaleimide, triiodobenzoate, KCN and heavy metals such as $Hg(NO_3)_2$ on the distribution of SH groups within the nucleus. The quantity of SH groups is determined by means of the Barrnett and Seligman histochemical procedure.

N-ethylmaleimide treatments of 4 and 24 hours at levels of 10^{-3}, 10^{-4}, and 10^{-5}M were given to onion root tips. The two lowest concentrations markedly decreased the over-all frequency of SH groups in the nuclei of the meristems. It appears that little damage is done to the chromatin at these concentrations. Mitosis is almost completely inhibited by the middle concentration and decreased in frequency by the lowest concentration. The highest concentration, on the other hand, killed the tissue.

KCN, a classic agent for uncoiling chromosomes, noticeably reduces the SH staining when given after fixation. Data will be presented showing the effect of this reagent on living root tips which have been subsequently fixed.

The effect of $HgCl_2$ depends upon its concentration. A saturated solution results in fixation but also leaves the nucleus in such a condition that it stains intensely with the SH procedure. A 0.05 M solution of $Hg(NO_3)_2$ fixes but also either dissolves protein or suppresses SH staining. A 0.005 solution of $Hg(NO_3)_2$ fixes some cells but not others and does not appear to affect SH groups differently than do other common cytological fixatives.

Hyde, Beal B. *See* Paliwal, R. L.

Hyun, Sin Kyu. Feasibility of Large-Scale Controlled Pollination as a Means of Mass Producing Hybrid Pine Seed. *Institute of Forest Genetics, Suwon, Korea.*

Because of the great economic potentialities of the hybrid of *Pinus rigida* × *Pinus taeda*, mass production of hybrid seed has been performed through large-scale controlled pollination. A 10-year-old pitch pine plantation, about 5 hectares in extent, has been employed as a maternal stand, and approximately 60,000 ovulate strobili were isolated with 30,000 pollination bags. Pollen of *P. taeda* were transported by air from the Institute of Forest Genetics, Placerville, Calif., U.S.A. Pollens from several individuals were mingled before using. Pollination was performed on 3 days as the ovulate strobili do not all reach the receptive stage simultaneously. On an average, 0.3 cc. (0.1 gr.) of pollen were used for each pollination bag to complete the pollination.

As a result of the pollination, 45,000 matured cones (133 litres in volume and 762 kg. in weight) were produced, and from these cones, 2,250,000 seeds (33.1 litres in volume) were produced. Among them, 1,305,000 (19.0 litres in volume) were sound seed and 945,000 (14.1 litres in volume) were hollowed seed, showing 58 per cent fertility. On an average, 43 sound seeds were produced from one pollination bag and from these seeds 25 1–0 seedlings

obtained, producing a total of 1,125,000 1–0 seedlings. These hybrid seedlings, through the test at the nursery bed as well as field planting up to 4 years old, have proven that the hybrid combines the cold resistance of pitch pine with the better form and growth of loblolly pine. Out of 100 hybrid seedlings 80 were large enough for field planting at the end of 1 year in the seed beds, whereas only 20 out of 100 ordinary pitch pine seedlings were large enough as 1–0 stock. On this basis, cost calculated per one item of planting stock was HW 2.65 for hybrid pine and HW 2.82 for ordinary pitch pine.

Thus, in Korea, the mass production of hybrid pine seed through hand pollination is feasible, not only technically but also economically, and a large-scale controlled pollination would be a short cut to the mass production of hybrid seed for reforestation until the seed orchard of parental species come into production.

IKEDA, YONOSUKE, and TOSHIAKI TAKAHASHI. Complementary Genes Controlling Alpha-methyl-glucoside Fermentation in Saccharomyces. *Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan.*

In contrast to the accepted belief that the fermentation of α-methyl-glucoside is under the control of one pair of alleles (*MG/mg*), the hybrid produced between a Carbondale haploid strain (rapid fermenter) and a haploid strain (non-fermenter), having been derived from a hybrid between a homothallic and a heterothallic Saccharomyces showed irregular segregation patterns with regard to the sugar fermentation. Three pairs of alleles, MG_1/mg_1, MG_2/mg_2, and MG_3/mg_3, were assumed as genes controlling the fermentation quantitatively, and haploid cultures carrying the genotypes (1) $mg_1\ mg_2\ mg_3$, (2) $MG_1\ mg_2\ mg_3$, (3) $mg_1\ MG_2\ mg_3$, (4) $mg_1\ mg_2\ MG_3$, (5) $MG_1\ MG_2\ mg_3$, (6) $MG_1\ mg_2\ MG_3$, (7) $mg_1\ MG_2\ MG_3$, and (8) $MG_1\ MG_2\ MG_3$ were actually recovered. The strains equipped with either (1) or (2), (4) or (6), (3), (5), (7), and (8) are non-fermenter, extremely slow fermenter, slow fermenter, medium fermenter, semi-rapid fermenter and rapid fermenter respectively.

The role of these genes in sugar fermentation and the identity or non-identity of some of these genes with maltose genes are under investigation.

INOUYE, NOBUO. *See* LERNER, I. MICHAEL

IRWIN, M. R. *See* BEDNEKOFF, A. G., *et al.*; STONE, W. H.

ISEKI, SHOEI. Lysogenic Conversion and Transduction of Genetic Characters by Temperate Phage *iota* in *Salmonella* Groups A, B, and D. *Department of Legal Medicine, School of Medicine, Gunma University, Maebashi, Japan.*

The production of somatic antigen 1 of *Salmonella* group A, B, and D is governed by temperate phage *iota*. However, in groups E, G, and H and in further groups which lack antigen 12, the relation between the production of antigen 1 and *iota*

phage is not clear. *Iota* phage, which induces lysogenic conversion with regard to somatic antigen 1 of *Salmonella*, can also transduce, like *epsilon* phage in *Salmonella* group E, such genetic characters as streptomycin resistance, nutritional factor, and flagellar antigen separately into the recipient strain. The transduction of characters can occur regardless of whether the recipient strain is easily lysogenizable or hardly lysogenizable. In the former case, the transduced bacteria turn lysogenic, acquiring somatic antigen 1, while in the latter, even after the transduction of characters, they generally remained sensitive without somatic antigen 1. The transduction of characters is induced by *iota* phage sometimes also into recipient strains which possess somatic antigen 1, but at much lower frequencies.

Ito, Takashi. A Kinetic Analysis of Immobilization due to Antigen-Antibody Reaction in *Paramecium caudatum*. *Biophysical Laboratory, St. Paul's University, Tokyo, Japan.*

Single antigen-antibody reactions are detected (operationally) by the immobilization of free-swimming *Paramecium caudatum*. Kinetic theories of immobilization of paramecium are developed, and experimental evidence is presented to verify them. The interesting character of immobilization is revealed by the analysis of the behavior of paramecia in intermediate cytoplasmic states (expressing two types of antigens simultaneously) in the presence of mixed antiserum.

Ives, Philip T. The Mutation Rate in *Drosophila* Sperm after Cobalt-60 γ Radiation. *Department of Biology, Amherst College, Amherst, Massachusetts, U.S.A.*

Spermatozoa from two-days-old inbred Oregon-R males were tested by Basc after exposure to Co-60 γ in doses ranging from 300 r to 12,500 r in intervals of 700 r to 2,500 r. For each of 7 doses a minimum of 1,202 X-chromosomes was tested and a minimum of 112 lethal chromosomes was observed. Plotted directly the data fit a straight line with a slope of 1.85% lethal chromosomes per 1,000 r. Tests of 342 lethal chromosomes from 12,500 r with *y ct ras f* showed 140 cases with reduced crossing-over, of which 131 gave subsequent genetic evidence (not confirmed cytologically) of translocations in 68 cases and of inversions within the X-chromosome in 63. In the 202 lethals with normal crossing-over there were only 3 instances of the lethal effect being due to the combined effects of two separable non-lethal genes. In 19 instances at least two separable lethal genes were found in the same chromosome. Probably many of the inversion chromosomes also carried two such genes. Thus individual mutational events probably occurred at a greater than linear rate of increase with increase in dose. Similar tests with 438 lethal chromosomes from 300 r showed only 2 instances of separable lethal genes and 23 cases with reduced crossing-over of which 9 were translocations and 14 inversions. These results are comparable to those in a previously published study of lethals produced by a genetic mutator except that in that study all of the 13 analysed cases of reduced crossing-over were inversions. (This work was done under Contract No. AT(30–1)–930 of the U. S. Atomic Energy Commission.)

JAAP, R. GEORGE, and B. L. GOODMAN. Use of Diallel and Triallel Mating Systems to Estimate Non-Additive Genetic Effects in Poultry. *Ohio Agricultural Experiment Station, Department of Poultry Science, Columbus, Ohio, U.S.A.*

Within independent 2 × 2 and 3 × 3 sets of sire × dam matings the total variance from two progeny per mating has been subdivided into that contributed by the sire, by the dam and by the sire × dam interaction. These subdivisions of the variance from 25 to 160 sets were pooled for each trait and components of the mean squares used to estimate the relative magnitude of non-additive effects in both a random-bred and a growth-selected population. Data were collected for weight of body and body tissues at hatching, growth, and variation in eggs and egg production of females. Accuracy in estimating the sire, dam and interaction components of the mean squares for the population has been compared by crude estimations of their fiducial limits and, for eight-week body weight, by the similarity of duplicate samples of matings.

On a basis of an equal amount of effort being expended in data collection, the triallels appear to provide estimates superior to those obtained from diallels. For body weight at eight weeks of age 225 to 270 matings in triallel composition gave estimated fiducial intervals equivalent to those from 600 matings in diallel form. Since heritability estimates from these large numbers of matings still appeared to have fiducial intervals of about 0.7 at a 90% confidence level, a larger number of progeny per mating should aid in reducing the excessive variation observed between sets in each of the sire, dam and interaction mean square components. In the random-bred population many of the quantitative traits were observed to be influenced by relatively large amounts of non-additive gene effects. This apparent excessive incidence of non-additive gene interaction may be attributable to the recent cross-bred ancestry of the random-bred population.

JAEGER, EUTERPE C. *See* CORDEIRO, A. R., *et al.*

JAHN, ALEXANDRA H. *See* CUANY, ROBIN L., *et al.*

JAMES, A. P. *See* NEWCOMBE, H. B., *et al.*

JAMES, ALLEN P. The Frequency Distribution of Radiation-Induced Mutants Affecting a Quantitative Character in Yeast. *Atomic Energy of Canada Limited, Chalk River, Ont., Canada.*

The spectrum of individual X-ray induced mutations that affect growth rate with varying degrees of severity has been studied in an inbred strain of yeast. Mutants were detected by observing differences in the growth rates of the four haploid segregants of single diploid cells. Differences in the rates of growth were measured by comparing the rates of change in the diameters of the microcolonies from the

four ascospores. The technique permits mutants to be detected where growth rates differ from those of non-mutants by only a small percentage. An exposure of 30,000 r produced recessive lethals and near-lethals (of all types) in one-quarter of the survivors. The ratio of these severe effects to the less severe but nevertheless detectable effects on growth rate (i.e., from about 90% reduction down to about 5% reduction) was approximately 1:1. The distribution of the different degrees of these non-lethal and near-lethal effects appears to be bimodal in yeast, as in *Drosophila*. A study of the dominance of the mutant genes is being undertaken.

JANICK, JULES. *See* PFAHLER, PAUL L.

JENKINS, B. CHARLES, and LAURIE E. EVANS. Some Alien Chromosome Additions to Common Wheat. *University of Manitoba, Winnipeg, Man., Canada.*

It has been suggested that the addition or substitution of chromosomes from related species may effect the transfer of germ plasm which would otherwise be impossible owing to the lack of chromosome homology between the species. Two such projects aimed at the production of whole chromosome substitutions from *Secale* and *Agropyron* to common wheat are in the advanced stages at the University of Manitoba. A preliminary step in the accomplishment of the substitutions is the production of the seven whole chromosome addition lines in the one case from Dakold winter rye to the winter hardy wheat variety Kharkov MC 22 and in the other from *Agropyron elongatum* chromosomes in the 56 chromosome derivative of the cross Chinese Spring $\times$ (Chinese Spring $\times$ *Agropyron elongatum*) F_{15} to the wheat variety Chinese Spring.

In both cases the procedure was to backcross the wheat parent to the "amphiploid" one or more times and to cytologically select monosomic and disomic addition plants in the subsequent generations. Several such 43 and 44 chromosome plants have been obtained and the extra chromosomes carried are being identified in two ways. First, the morphology of the plants is compared and gross differences are regarded as being the effects of the addition of different alien chromosomes. Secondly, the supposedly different disomic plants are diallel crossed and the cytological behavior of the extra chromosomes in the F_1 generation is used as a criterion for positive identification.

The addition lines are expected to furnish information as to the location of genes in the various chromosomes of rye and *Agropyron elongatum*. They will also be used as a means of making systematic substitutions of the alien chromosomes into the Kharkov MC 22 and Chinese Spring varieties.

JENKINS, J. A., and B. O. BERG. Inheritance of Fruit Size and Shape in the Tomato. *Department of Genetics, University of California, Berkeley, Calif., U.S.A.*

A method for dealing with the individual loci of continuously varying character differences, originally suggested by studies of carotenoid differences in tomato fruits

(Jenkins and Mackinney, 1953, 1956), was applied to a study of fruit size and shape inheritance. Subsequently, we discovered that similar procedures were outlined by Comstock and Robinson (1952) in their type III experiment for estimating the degree of dominance.

In 1949 Lindstrom's *d-p-o-s* (dwarf, peach fruit, oval fruit, compound inflorescence—linkage group I) was crossed with MacArthur's *f-a-j-lf* (fasciated fruit, green stem, jointless pedicel, leafy inflorescence—linkage group V). In addition to the two parents, the following hybrid cultures were grown in a randomized field planting in 1955: F_1, F_2, backcrosses of F_1 to both parents, an F_3 and testcrosses to both parents (three cultures) from each of 28 previously grown F_2 plants.

On a logarithmic scale segregation at the major loci *o* and *f* contributed 30% and 50% of the total additive genetic variance. The remainder was attributed to modifying genes. There was no interaction between the major loci. Each exhibited partial dominance, but in a different direction. The method of estimating average dominance outlined by Comstock and Robinson (1952) gave a more satisfactory agreement with the data than that outlined by Fisher, Immer and Tedin (1932) and elaborated by Mather (1949).

From the later generations of this hybrid we have isolated the four homozygous genotypes, *OOFF, OOff, ooFF* and *ooff*, in a relatively uniform background. When all four tester lines are crossed with a new variety, the locule number of the F_1 hybrids may be used to determine what alleles the new variety has at the *o* and *f* loci and if it has new alleles at other major loci determining variability in locule number.

JOHNSON, B. LENNART. Linkage on the S Chromosome of *Matthiola incana*. *University of California, Los Angeles, Calif., U.S.A.*

The eversporting mechanism *1S/+s* described in *Matthiola incana* consists of a chromosome carrying a pollen lethal, *1*, closely linked with the gene *S* for single flowers and of a homolog carrying *s* for double flowers. The heterozygous condition of *s* perpetuated by this mechanism results in 46 single : 54 double-flowered plants in each successive generation, the deficit of singles being due to the sublethal effects of *1* on ovules or zygotes.

Disregarding the very low *1-S* crossover frequency, linkage values between *S* and other genes have been estimated by methods using half of the F_2 phenotype classes.

Considering complete non-function of *1* pollen and a calculated viability coefficient of v = .83 for eggs or zygotes carrying *1*, the phenotype probabilities from the linked genes *S* and *W* in terms of the crossover value, p, become:

	Coupling	Repulsion
SW	$v/1+v\ (1-p+p^2)$	$v/1+v\ (1-p+p^2)$
Sw	$v/1+v\ (p-p^2)$	$v/1+v\ (p-p^2)$
sW	$1/1+v\ (2p-p^2)$	$1/1+v\ (1-p^2)$
sw	$1/1+v\ (1-2p+p^2)$	$1/1+v\ (p^2)$

from which the linkage value may be calculated by conventional methods using all of the F_2 data.

The probability of the *Sw* crossover class in coupling phase at all values of p is less than half of the *sW* class. In repulsion phase both of the *Sw* and *sw* crossover classes are small—neither one exceeding 14% of the total phenotypes at p = .50.

Although *M. incana* tends towards homozygosity, owing to nearly complete self-fertilization, the area of the *S* chromosome adjacent to *1* tends to harbor deleterious recessives in the heterozygous condition. Such recessives occurring on the *1S* homolog remain unexpressed until a crossover takes place. On the $+s$ homolog they are also perpetuated but are expressed each generation in the sterile doubles. This area contains 3 recessives which affect chlorophyll or plastid production. Farther from *S* lie genes affecting flower color and plant habit.

JOHNSON, L. P. V., and RUSTEM AKSEL. Inheritance of Yielding Capacity in Barley. *Department of Plant Science, University of Alberta, Edmonton, Alta., Canada.*

The objectives are: (*a*) discovery of parental combinations that produce high-yielding progenies; (*b*) determining whether these high-yielding combinations are apparent in F_1; and (*c*) genetic analysis by biometrical techniques. Fifteen barley varieties were crossed unidirectionally in all combinations (105 crosses). The F_1 materials of a 9-parent diallel (36 crosses) were greenhouse grown in 1955–6 and the remaining material (69 crosses) in 1956–7. Each cross was represented by 40 plants (10-plant rows in 4 replications). The F_2 materials of the complete 15-parent diallel (105 crosses) were field grown in 1957, using a simple, 11×11 lattice (4 replications). Each plot consisted of four 16½-foot rows.

Yields of F_1 and corresponding F_2 populations gave correlation coefficients of $r = +0.4815^{**}$ for 36 crosses or $r = +0.2735^{**}$ for 105 crosses. The genetic analyses of the 36 crosses + 9 parents showed (*a*) dominance present but (*b*) not unidirectional, and (*c*) significant asymmetry at loci exhibiting dominance. Although the heterogeneity of Wr-Vr was not significant (error value was rather high), analysis of the (Vr, Wr) graph suggests possible non-allelic gene interactions; genotypic-environmental interaction is probably also present. All Vr, Wr points were below the line of unit slope through the origin; the actual Wr/Vr regression line is less than unity and not significant. The general picture is one of over-dominance.

Analytical results of the F_2 (105 crosses + 15 parents) are roughly similar to those of F_1. The heterogeneity of Wr-Vr is nearly significant at the 0.05 level. The averages of the Wr/Vr points lie below the line of unit slope through the origin: i.e., the F_2 data also show over-dominance.

JUCCI, CARLO. Physiogenetic Researches on Heterosis in Silkworms (*Bombyx mori*). *Centre of Genetics, National Research Council, Istituto "L. Spallanzani," Università, Pavia, Italy.*

The investigation of heterosis in domestic animals, such as silkworms, may have interesting results not only for breeding—more efficient utilization of hybrid vigour —but also for theory, leading to: (1) a deeper physiogenetic understanding of interactions between alleles of the same locus and alleles of different loci—exploring overdominance and complementary effects; and (2) a more adequate factorial analysis of processes both for adding beneficial effects of dominant genes and for buffering unfavourable effects of recessive genes.

Study of the phenotypic expression of heterosis such as increase in resistance, aspecific and polyvalent, and increase in fertility—the best advantage of hybrid

vigour in double hybrid Japanese silkworms, as in double hybrid American maize—is interesting also for a better understanding of the role of heterosis in nature.

In nature gains in resistance—to unfavourable, biotic and physical, environmental conditions—and in fertility contribute most efficiently to the superiority of the heterozygote and influence, through it, genetic structure of populations, breeding system and evolutionary pattern.

Besides resistance and fertility, the following items have been investigated in order to clarify the physiological aspects of hybrid vigour: (1) intraovular and larval development in F_1 hybrids studied as weight growth and oxygen consumption; (2) silk gland's growth and secretion in F_1 hybrids and respiratory activities of the glandular epithelium; (3) resistance of F_1 hybrids to DDT and other chlorinated insecticides.

The comparison with parental breeds has been effected in couples of reciprocal hybrids, so as to allow the study of maternal prevalence in the earlier stages of development and to discover cytoplasmic factors of heterosis if any coexist with chromosomic ones.

For a better analysis of the heterosis effect some of the F_1 hybrids have been compared with parental breeds in series of families having, two by two, the same father and different mothers.

JUDD, B. H. An Analysis of the Subdivisions of the White Region in *Drosophila melanogaster*. *University of Texas, Austin, Texas, U.S.A.*

The spatial relationships of several of the mutants of the pseudoallelic white series in *Drosophila melanogaster* have been examined. There are at least three separate loci which may be distinguished. They may be represented by the mutants Brownex (w^{Bwx}), apricot (w^a) and white-1 (w^1), located from left to right in the order named.

Some mutants of the series cannot be located with certainty in relation to these three loci. The mutant blood (w^{bl}), for example, will not recombine with either w^{Bwx} or w^a but apparently is located to the left of eosin (w^e), which in turn appears to be at the w^1 locus. The mutant buff (w^{bf}) recombines with w^{Bwx}, w^a and w^e but cannot be located exactly because of the appearance of recombinant classes which contradict an assignment to a specific locus (e.g., from y^2 w^a *spl ec*/w^{bf} females, the exceptional crossover types are *w spl ec* and y^2 *w*). Such results may indicate that w^{bf} occupies a fourth locus in the region.

From some heterozygous combinations different exceptional types result from crossovers in the same direction; for example, w^+ *spl ec* and *w spl ec* types are recovered from y^2 w^{Bwx} *spl ec*/w^h females. In this and similar cases it has been found that the crossover class of white phenotype is not the double mutant.

The spatial relationships of the pseudoallelic loci and the origin of some of the recombinant classes will be discussed.

JUGENHEIMER, ROBERT W. Performance, Uniformity, and Practicability of Various Types of Corn Hybrids. *University of Illinois, Urbana, Ill., U.S.A.*

The balanced nature of this experiment permitted studying the relative performance and uniformity of plant and ear characteristics of various types of corn hybrids.

Four inbred lines of corn, Hy, L317, WF9, and 38-11, were combined in all-possible combinations to produce 6 single crosses, 12 three-way crosses, 12 single-backcrosses, 12 backcrosses, 3 double crosses, and 6 top crosses. The theoretically homozygous inbred lines were more variable than the hybrids in ear weight, ear length, and ear height, traits greatly influenced by the environment. The inbred lines were very uniform in number of kernel rows, a trait determined primarily by genetic factors and affected very little by the environment. Highest yields and greatest uniformity were obtained from the single cross, three-way cross, single-backcross, and backcross hybrids.

Single cross hybrids were slightly higher yielding and more uniform than other types of hybrids. The relatively high cost of producing seed of single crosses limits their use to situations where extreme uniformity is important. Double crosses (the most widely grown type of hybrid) and top crosses were considerably lower yielding and more variable in plant and ear traits than the other types of hybrids. Of the 12 single-backcross hybrids 8 yielded more grain than the best double cross. The best single-backcross hybrid yielded 9 bushels (nearly 10%) more than the best double cross hybrid.

Seedsmen are interested in hybrids with greater uniformity and performance than presently used double cross hybrids. Single-backcross hybrids appear to offer commerical utilization. They produce higher grain yields, contribute greater uniformity of plant and ear, and are practical to produce, inasmuch as the seed is grown on a vigorous single-cross parent.

KÄFER, ETTA. Analysis of a Translocation by Means of Mitotic Recombination in *Aspergillus nidulans*. *Department of Genetics, McGill University, Montreal, Canada.*

After ultraviolet irradiation of a standard biotinless (*bi*) strain of the fungus *Aspergillus nidulans* a cholineless (*cho*) mutant was isolated. In the course of mitotic localization of this mutant, it became evident, that the new *cho bi* strain carried a chromosomal aberration involving linkage groups I and VII. Mitotic haploidization in diploid fungus strains produces segregants which are recombinant for whole chromosomes, that is, similarly to results obtained by the *Drosophila* inversion technique, markers of the same chromosome show complete linkage.

The mutant *cho* was crossed to standard strains with various markers of linkage groups I and VII and meiotic recombinants were then tested by means of mitotic haploidization. The original *cho bi* strain and some of these meiotic recombinants showed complete linkage of all markers of linkage group I with those of linkage group VII in mitotic haploids, while others showed normal linkage of only the markers within each group. In analogy to similar findings in *Drosophila* it was concluded, that a translocation between the two chromosomes is present in the original *cho bi* strain, which can be separated from *cho* by meiotic crossing over. Very close linkage (4% crossing over) of the translocated piece to the most distal marker on the left arm of linkage group I (a suppressor of adenine-20) was found and results from mitotic crossing over confirmed, that a substantial chromosome piece of linkage group VII is attached in this position. Whether this is a case of a reciprocal translocation and what the exact size of the translocated piece(s) is, cannot be decided, as both available markers of linkage group VII show practically

50% recombination with the translocation. New markers in suitable positions and cytological analysis may give further information on these points.

KÁLLAY, I. *See* GYÖRFFY, B.

KALTER, H. Studies on the Inheritance of Susceptibility to Congenital Malformations Produced by Maternal Riboflavin Deficiency in Mice. *Children's Hospital Research Foundation, Cincinnati, Ohio, U.S.A.*

Intra- and inter-strain crosses were made with members of several inbred mouse strains. (Results from various backcrosses now being made may also be presented.) At 9⅝ days after conception, females were given for 4 days a riboflavin-antagonist, galactoflavin, incorporated into a riboflavin-deficient diet. Young were removed before term and examined grossly and histologically for congenital malformations.

Numerous skeletal, encephalic, and esophageal malformations occurred in offspring of intra-strain crosses (in whom a survey of effects of riboflavin deficiency on embryonic development was made). Young from inter-strain crosses were scored for several malformations only—cleft palate, syndactyly, ulnar abduction of hand, absent esophagus, and brain anomalies.

Surprising results ensued from a study of frequencies of these malformations in F_1 offspring as related to frequencies in parent strains. In general, it appeared that (1) where frequencies in parent strains were relatively high and not statistically different from each other, F_1 frequencies were greatly lower than those of the parent strains; and (2) where the parent strains were different, one having high, the other low frequency, the F_1's had frequencies on the order of that of the resistant parent strains. The first finding reveals the stabilizing power of hybridity and the second partly indicates the dominance of factors responsible for resistance.

KAMRA, OM P., and R. A. NILAN. Anatomy of the Multiovary Mutant in Barley. *State College of Washington, Pullman, Wash., U.S.A.*

The multiovary (*mo*) mutant of barley is characterized by the transformation of stamens into pistils. It was induced by irradiation and behaves as a simple recessive (Moh and Nilan, J. Hered. *54*: 183–188). The present paper is a report of a study of the anatomy and the embryo sac development of the mutant florets.

The transformation of stamens into pistils appears to take place very early in the floret development. It is complete in most florets, whereas it is only partially complete in the remainder.

A floret of normal barley has two lodicules. In the mutant plant, however, the florets of median spikelets have one lodicule and those of the lateral spikelets have two. In general, the mutant florets appear to contain more vascular tissue than normal florets.

The embryo sac appears to be normal (the Polygonum type) with six antipodals. A frequent abnormality is the formation of a T-shaped tetrad instead of a linear tetrad. Ovules of the mutant florets are naked or enclosed, while those of normal barley are always enclosed.

Since the mutant florets are variable as to the degree of transformation of stamens, number of lodicules, and covering of the ovule, it is concluded that the gene *mo* has a low expressivity.

Further studies of this mutant should be valuable for an understanding of sex determination and sex reversal in higher plants.

KANEHISA, TAKEHARU. A Study of Some Metals in "Melanotic Tumor" Strains of *Drosophila*. *Osborn Zoological Laboratory, Yale University, New Haven, Conn., U.S.A.*

Genetic and physiological studies have shown that the character "melanotic tumor" (*tu*) in *Drosophila* is intimately related to a tryptophane metabolic pathway (Kanehisa, 1954, 1955, 1956, 1957). The present investigation deals with the probable relationships of some metals to tumor formation, tryptophane metabolism and melanization. Eggs of the strains *tu*, *bw tu*, *v tu*, *tu st*, ±, *bw*, *v*, and *st* were placed on the synthetic medium of Sang, with some variation, and pupae were analysed for Fe, Cu, Co, Ni and Mo content by the methods of Sandell and Kikkawa, with some technical improvements. Viability and incidence of melanotic tumor were also studied. Results may be summarized as follows: (1) the amounts of Cu, Fe and Co accumulated in pupae are relatively lower in *tu*-strains than in non-*tu*-strains; (2) the amounts of Fe and Co are higher in *tu* and *bw tu* pupae than in *tu*-strains containing gene related to tryptophane metabolism, although the reverse relationship holds for the amounts of Cu present; (3) Viability of *tu*-strains is relatively lower than that of non-*tu*-strains; (4) Incidence of melanotic tumors and viability of *tu*-strains are higher in *tu*-strains which contain gene related to tryptophane metabolism than in *tu*-strains without such gene. The relationships of tryptophane metabolic pathway, tumor metabolism and melanin formation will be discussed in the light of present results and the data previously published.

KANEHISA, T., and D. F. POULSON. Nucleo-Cytoplasmic Interaction in the Control of Metal Accumulation in Two Strains of *Drosophila melanogaster* and Their Heterozygotes. *Department of Zoology, Yale University, New Haven, Conn., U.S.A.*

Advances in knowledge of metal metabolism together with evidence for its genetic control in *Drosophila* (Poulson and Bowen, Exp. Cell Res., Suppl., 2: 161–180; Poulson *et al.*, Proc. Nat. Acad. Sci. *38*: 912–921; Poulson, Genetics *40*: 590; Kikkawa, *et al.*, Science *121*: 43–47) have focused our attention on the responses of the *ebony* series of alleles to the level of copper in the medium on which the larvae are raised. Striking differences in the survival of $ebony^{11}$ as compared with *ebony* and *Oregon R* strains led to a combined study of metal accumulation and survival in $ebony^{11}$, *Oregon R*, and their heterozygotes at different levels of copper in the medium.

To simplify the nutritional situation the experiments were carried through on Sang's (J. Exp. Biol. *33*: 45–72) chemically defined medium under aseptic conditions. Two levels of iron and three levels of copper were employed. In each creamer 50 eggs were placed on 5 ml. of medium and maintained at 25°C and

nearly 100% humidity to prevent evaporation. Comparisons were made between homozygotes (e^{11}/e^{11} and $\pm/\pm$) and both types of heterozygotes ($e^{11}/\pm$ and $\pm/e^{11}$). Survival was measured as the percentage adult emergence. Copper, iron, nickel, cobalt, and molybdenum were determined analytically using standard procedures on samples of 150–200 pupae.

The survival data are in agreement with those on standard medium with live yeast although on the defined medium the levels of survival are lower for the toxicity threshold is also lower. While there is some survival of wild type and heterozygotes on the standard medium up to 300 μg. Cu/g., a concentration of 100 μg. Cu/g. in the defined medium is toxic to all 4 genotypes. The order of survival is the same on the defined medium with 60 μg. Cu/g. and on standard medium with 100 μg. Cu/g., e.g., $\pm/\pm > e^{11}/\pm > \pm/e^{11} > e^{11}/e^{11}$.

Metal analyses gave these interesting results. Cu accumulation is significantly higher in e^{11}/e^{11} than in $\pm/\pm$ larvae at the three Cu levels employed. There are even larger differences between the two types of heterozygotes which also differ from the homozygotes. There are striking differences in Fe content of larvae among the different genotypes at different Cu levels: $\pm/\pm$ larvae contain nearly twice as much Fe as e^{11}/e^{11} on medium with no added Cu, but drop to very low levels at higher Cu concentrations while e^{11}/e^{11} show a marked increase in Fe. With no added Cu $\pm/e^{11}$ larvae accumulate twice as much Fe as $\pm/\pm$ and three times as much as e^{11}/e^{11}. At higher Cu concentrations the Fe content of $\pm/e^{11}$ drops off, but is still high in relation to $\pm/\pm$. With no added Cu $e^{11}/\pm$ larvae have the same Fe content as e^{11}/e^{11}, but with added Cu this falls off, paralleling $\pm/\pm$. When the Fe concentration in the medium is halved the differences among the genotypes with regard to both Fe and Cu accumulations are much less marked and the survival of e^{11}/e^{11} is further lowered in relation to other genotypes. Analyses for Ni, Co, and Mo were also made in one experiment. There appeared to be no significant change in Ni content with higher Cu concentrations, although there was an increase in Mo content in all genotypes and a decline in Co content.

The differences in patterns of Cu and Fe accumulation between the two types of heterozygotes provide an excellent example of nucleo-cytoplasmic interaction and are being investigated further from this point of view.

(Work supported by a grant from the National Science Foundation.)

KANG, YUNG SUN. A Study of Korean Population Genetics: Vital Statistics of Different Occupational Groups in the Korean Population. *Seoul National University, Seoul, Korea.*

Data have been obtained on the vital statistics which are correlated with population genetics for a Korean population of 220,456 who are resident in several areas.

For 2 years, from 1955 to 1957, measurements were made of the variations of the following factors. Birth rate of the Korean population is 16.31 per 1,000, in general, but among those engaged in heavy labour, this is higher, 18.86, as is commonly found in most populations.

It is well known that the Korean population has the highest sex ratio at birth compared with other populations. The ratio found here is 113.92 males per 100 females.

The death rate is slightly lower than the birth rate, 14.73 per 1,000. Comparing

the rate of the group engaged in mental work with that of the group engaged in physical work, scarcely any difference could be found between the two groups.

In this research, 318 pairs of twins were recorded. Of these 66.7% were monozygous twins, and their sex ratio was higher than the rest of the population: 126.33 males per 100 females. The proportion of sexual combination is ♂♂ 2.85: ♂♀ 1.00: ♀♀ 2.16.

From 1953 to 1956, the author studied 1,364 marriages which included 65 cousin marriages. The frequency of cousin marriages is thus 4.76%.

KAPLAN, WILLIAM D. The Sterility Component of X-Ray Induced Dominant Lethals in *Drosophila melanogaster*. *City of Hope Medical Center, Duarte, Calif., U.S.A.*

In *Drosophila melanogaster* dominant lethals are determined by egg hatchability tests. Brooding techniques, the mating of treated and control males to a succession of virgin females, permit the determination of mutation rates in sperm that were successively younger at the time of irradiation. However, *Drosophila* offers no direct way to distinguish between eggs that have failed to hatch because they remained unfertilized and eggs that have failed to hatch because development of the embryo has broken down as a result of an induced genetic event. For this reason Kaplan, Tanaka and Tanaka (Rec. Gen. Soc. Amer. *25*: 649) carried out a preliminary parallel genetic and cytological study. A sample of eggs from each dominant lethal determination was fixed and sectioned and studied for evidence of sperm entrance. It was found that unfertilized eggs contribute significantly to the dominant lethality rate based upon hatchability tests. The present report presents data on sterility obtained when the X-ray dosage and the mating intensity (number of females per male) are varied. Three X-ray dosage levels, 500, 1,500 and 2,500 r units, were used. At each dosage level three broods each of one female per male, two females per male, and three females per male were carried out. Each male was permitted to mate over a period of nine days, three days per brood.

The data show that sterility (failure of sperm to enter an individual egg) increases with dosage and is highest in the third brood; but brooding itself is no cause of sterility. The high sterility observed in the treated third brood fertilizations (sperm that were spermatocytes or younger at time of irradiation) seems to be a combination of factors produced by the radiation and the utilization of sperm.

KATSUO, KIYOSHI. *See* MIZUSHIMA, USABURO.

KAUDEWITZ, FRITZ. A New Type of Mutation-Delay in Bacteria. *Max-Planck-Institut für Virusforschung, Tübingen, Germany.*

UV-irradiated cells of *Salmonella typhimurium*, auxotrophic strain lys-1, give rise to prototrophic "reversions." Three hours after plating the non-mutated cells start logarithmic growth. As demonstrated by respreading, the number of potentially mutated cells per plate, however, remains constant up to 5 hours and 40

minutes after. This delay could be due to (*a*) a prolonged lag phase of mutants, (*b*) segregation of several nuclei per cell or homologous genetic subunits per nucleus, only one of which is mutated, (*c*) the formation of filaments, or (*d*) the linear transfer of the character "potentially mutated." Negative experimental evidence favours (*d*). Between 0 and 5^{40} or 3 and 5^{40} respectively penicillin kills non-mutated and potentially mutated cells with the same efficiency, thus excluding (*a*). Cells washed off the plates at 5 hours are separated by centrifugation and filtration into fractions, all of which have the same ratio of mutant/non-mutant cells, thus excluding (*c*). Higher levels of lysin in the minimal medium between 3 and 5^{40} increase the number of generations from ¾ to 4½. The increase of mutated cells however unchanged starts at 5^{40}, thus excluding (*b*).

The genetic manifestation of the "reversion" is highly temperature-sensitive: temperatures of 34°C and lower give 100% yield of mutants which is gradually decreased to 0% by increasing the temperature to 36.5°. Various lys-1 "reversions" usually differ in colony size. The prototrophic character of only about 10% is transducable to lys-1 cells. Transduction analysis proves them to be due to suppressors. X-ray radiation of transducing phages being synthesized in transducable reversions reveals that the target size of the genetic material causing the prototrophic character varies greatly in different "reversions" and is bigger than a gene locus.

The same degree of temperature sensitivity of genetic manifestation, rare transducability and delayed increase in number is shown by spontaneously arising lys-1 "reversions" and was found in the reversions of 11 from 90 auxotrophic mutants of *Salmonella typhimurium* and *Escherichia coli* (no transduction analysis), thus demonstrating the generalisation of the phenomenon.

Experimental data are in agreement with the following working hypothesis. By the mutational event part of the genetic material is separated from the linkage group. Having lost its reproducibility it is transferred for some time linearly. Its reincorporation may lead to a variety of rearrangements which, as nongenic suppressors, cause quantitatively different prototrophic characters. Most of these rearrangements are too large in size to be transducable.

KEEP, ELIZABETH. *See* KNIGHT, R. L.

KHITRINSKY, V. Controlled Heredity Changes Aimed at Transforming Spring Varieties into Winter Crops Seen as a Plant-Breeding Method. *Genetics Department, All-Union Genetics and Plant-Growing Institute, Odessa, U.S.S.R.*

Seeds of several varieties of spring wheat, previously checked for genetic homogeneity were sown in the open in the pre-winter period (from late November to December). The generations to follow were sown at about the usual time of winter wheat sowing in the given region (September).

Under conditions of autumn sowing spring wheat varieties (Lutescens 1163, Odesskaya 13, Milturum 274) underwent several changes in morphology. These included the creeping type of stem typical of winter varieties instead of the usual erect type, a decrease in length and spiral twisting of the leaves and an increase in the number of plants with glabrous leaves. These changes were hereditary and

were preserved in the progeny with considerable stability. Physiological characteristics affected by autumn sowing included an increase in the dry matter content, an increase in the sugar percentage, an increase in the water retaining capacity and a lengthening of the vernalization stage. These changes also proved to be hereditary and preserved with great stability in the second generation.

The modified strains of rye and wheat yielded an increased number of frost-resistant plants with winter crop characteristics according to the quality and quantity of assimilated light. The greatest number of such plants was developed in the groups which were given additional night lighting with incandescent lamps.

Modified Odesskaya 3 wheat showed a changed total time of ear formation. Experiments were also undertaken to assess resistance to brown rust and stinking smut of modified strains. Grain yield for the modified strains was generally below that of the standards, although in certain strains it was increased. Most of the lines of modified wheat possess high qualities of grain, flour and bread.

The method of controlled heredity changes aimed at transforming spring varieties into winter crops has been worked out to a degree when it can be successfully used in practical plant-breeding.

KIDWELL, J. F., and H. J. WEETH. The Effect of Nutritional Environment on Selection for 70-day Body Weight in the Laboratory Rat. *University of Nevada, Reno, Nev., U.S.A.*

Four inbred lines of laboratory rats were used to form a four-way cross. Four lines, consisting of 10 males and 10 females each, were randomly established from this cross. Two of the lines were maintained on a standard laboratory ration and two on a ration consisting of 35% ground alfalfa and 65% standard laboratory ration. One line on each nutritional regimen was selected for 70-day body weight and the other selected at random. Random mating was practised in all lines, except that full sib matings were prohibited. Each litter was reduced to 6 at 5 days, with an attempt to retain 3 males and 3 females. Most litters contained 6 individuals; there was none with less than 4. Litters were weaned at 28 days and maintained on the test ration until 70 days. Selected individuals of all lines were put on the standard ration at 70 days. Females were bred at 100 days and produced only one litter. This paper summarizes the results of the first 5 generations. The data consist of 28- and 70-day body weights. The four-way cross generation is designated as the zeroth generation; the next as the first selected generation, etc.

In all lines both 28- and 70-day weight decreased during the first and second selected generations. In the selected lines selection differentials were positive and significant. In the random lines there were no significant differences between the mean of the entire generation and the parents of the next generation. In the third, fourth and fifth generations a positive selection response was observed. The selected line on the standard rations has surpassed the four-way cross while the selected line on the roughage ration is at the level of the four-way cross. The random lines have remained at about the level of the first selected generation. These observations suggest heterosis in the four-way cross, probably as a result of favorable epistatic combinations. They also suggest that as favorable epistatic combinations are broken up in successive generations there is an increasing effect of genes that act in an additive fashion.

Heritability estimates have been computed by three methods: regression of off-

spring on midparent, regression of offspring on parent, and correlation between full sibs. The estimates are quite erratic and do not reflect actual selection response. Small sample size may be invoked as an explanation but is hardly adequate. A completely satisfactory solution is not immediately apparent.

KIHARA, H., K. YAMASHITA, and M. TANAKA. Some Aspects of the Genomes of 6*x* Species in *Aegilops. Biological Laboratory, Kyoto University, Kyoto, Japan.*

A classification of the genus *Aegilops* by means of genome-analysis was published in 1954 (Cytologia *19*). At that time the study of the 6*x* species, *Ae. triaristata, Ae. crassa* and *Ae. juvenalis,* was still incomplete. Recently, we have obtained enough data on the basis of the morphological as well as genome-analytical analyses to draw the conclusion that the third genome of the hexaploid *Ae. triaristata* is a modified M and that of *Ae. crassa* is D. The genome-type of *Ae. juvenalis* was also ascertained as $DDC^{u}C\ {}^{u}M^{j}\ M^{j}$.

KIKKAWA, H. Genetic Analyses of the Resistance to Parathion in *Drosophila melanogaster. Department of Genetics, Faculty of Medicine, Osaka University, Osaka, Japan.*

Genetic studies on the resistance to chlor-organic insecticides such as DDT and BHC have been performed extensively throughout the world, whereas studies on organic phosphorous insecticides have been few and incomplete. The test method adopted here was mainly the "larval test." A certain number of late first instar larvae (50–100) were transferred to the medium containing parathion, and the rate of emergence was studied. Very similar results were obtained by the "adult test." The results of the genetic analyses are as follows: (1) The resistance to parathion is mainly controlled by a dominant gene locating near 64.5 on the second chromosome. Any susceptible second chromosome which received the portion involving this dominant gene by means of double crossing-over shows the resistance not only to parathion but to other insecticides such as DDT and BHC. Therefore, this may be the same or pseudoallelic with that of resistance to DDT and BHC reported by Tsukamoto and Ogaki. (2) In addition to this major gene, the parathion-resistance is also controlled by a dominant gene locating near 50 on the third chromosome. But the effect of the third chromosome is very weak. The gene may be identical with that for nicotine-resistance. Furthermore, where two strains differing extremely in resistance are used, an effect of a sex-linked gene is recognized under certain condition. (3) Of great interest is the fact that in a high parathion concentration, a phenomenon of maternal effect is clearly observed. Moreover, some interesting facts in relation to parathion-resistance were found by my co-workers, and will be reported.

KIMBALL, ROBERT F. The Postirradiation Period and Mutation in *Paramecium. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

Since the process by which radiation produces recessive lethal mutations in *Paramecium* is relatively slow, it can be modified by treatment after irradiation. The

hypothesis has been advanced that the mutational process is spontaneously reversible when initiated during the first half of the interdivision interval but is irrevocably completed at about the middle of the interval. Procedures that retard growth, provided that they do not interfere with the reversal of mutation itself, should decrease mutation by allowing extra time for reversal.

The present paper points out the variety of apparently unrelated features of mutation in *Paramecium* that can be understood with this hypothesis. First, the diversity of procedures that decrease mutation is explained; all slow growth and need have nothing else in common. Secondly, the increase in apparent sensitivity during the first half of the interval is explained; there is more time for recovery when radiation is given early. Thirdly, several differences between X-rays and ultraviolet become understandable. Ultraviolet markedly slows growth in the dose range used; X-rays do not. Therefore ultraviolet probably acts both as a mutagen and a "recovery" agent, whereas X-rays act as a mutagen only. Fourthly, prolonged starvation before irradiation decreases the amount of mutation produced by X-rays; this is most easily explained by the effect of starvation on growth after irradiation.

Work with a number of organisms has established that mutational change, in the broad sense, is often a relatively slow process that can be influenced in a variety of ways after irradiation. The work with *Paramecium* suggests the importance of this fact in understanding various features of the mutational process and emphasizes the important influence that effects on cell growth may have.

KING, ROBERT C. *See* BENDER, HARVEY A.

KLOEPFER, H. WARNER. Progress Report on Study of a Type of Progressive Juvenile Dementia with Oligophrenia and Erythema. *Department of Anatomy, Tulane University, New Orleans, Louisiana, U.S.A.*

Seven cases (4 males and 3 females) of progressive juvenile dementia with oligophrenia and erythema occurred among 22 offspring in 4 families. The 8 parents are first or second cousins with a common ancestor. Inheritance is by an autosomal recessive gene with 100% penetrance. This syndrome is being studied from the standpoint of neuropathology, neurology, dermatology, biochemistry, and ophthalmology.

Each affected individual is believed to be normal until one or two months of age when severe blistering occurs from exposure to sunlight. Erythema gradually subsides by the age of 5 or 6 years when growth (measured by stature and weight) ceases uniformly in all parts of the body. Mental age does not progress beyond the level of an imbecile. Immediately after or during a normal adolescence progressive degenerative dementia accompanied by a loss of vision sets in which lasts until death occurs between the ages of 20 and 31. For several months prior to death patients are completely blind, deaf, and bedfast idiots.

The various stigmata in each case seem to be expressed in a very similar manner and at a similar age. This syndrome does not seem to have been described previously. In a preliminary neuropathological report on one patient John Moossy,

(neuropathologist, Louisiana State University School of Medicine) states: "In the limited material available there is severe brain damage consisting of loss of nerve cells in the cerebral cortex and other areas, widespread ferrugination of cortical capillaries and capillaries within the putamen, large areas of subcortical demyelination with astrogliosis, alterations in the dentate nucleus of the cerebellum and gliosis in the cerebellar white matter, and excessive accumulations of lipochrome probably representing some degree of pigmentary degeneration in the nerve cells of the medulla, pons, dentate nucleus of the cerebellum and some areas of cerebral cortex."

KLOEPFER, H. WARNER. *See* WATT, DEAN D.

KNIGHT, R. L., ELIZABETH KEEP, and J. B. BRIGGS. Resistance in the Red Raspberry to *Amphorophora rubi* (Kalt.). *East Malling Research Station, East Malling, England.*

Breeding against raspberry viruses at East Malling is concentrated mainly on vector resistance, since several of the numerous viruses that attack the raspberry in Great Britain are carried by the aphid *Amphorophora rubi*. The resistance of the variety Baumforth A to *Amphorophora* is controlled by a single dominant gene A_1, which is linked with the normal allele of a semi-lethal gene, *fr*. The cross-over value of these two loci is approximately 3.3%. The gene A_1 confers high resistance both to seedling plants in the insectary and to mature plants in the field.

In the course of the work, evidence for the existence of hitherto undescribed biologic races of the aphid was obtained, only one of which was partially successful in colonising A_1 plants. A source of resistance to this race has been found.

KNIGHT, ROBERT J., Jr. The Adaptive Significance of Variation in Response to Dark Period in *Ipomoea*. *Blandy Experimental Farm, University of Virginia, Boyce, Va., U.S.A.*

Four species of *Ipomoea* and one artificially produced amphiploid, assembled from a number of locations at differing latitudes in the Northern Hemisphere, were grown in northern Virginia, at 39° north latitude, under conditions of natural day-length. In the short days of winter or of early spring or the shortening days of midsummer, those from the northernmost sources began blooming before those originating farther south. There was a trend of declining sensitivity to lengthening nights, demonstrated by a tendency to remain longer in vegetative growth, as parent stocks originated at lower latitudes. Hybrids between "long-day" and "short-day" taxa in two species appeared to demonstrate the genetic dominance of "short-day" to "long-day" bloom response. A small F_2 population appeared to show segregation for flowering behaviour when grown in an artificially maintained dark period of ten hours. In *Ipomoea*, populations which have invaded relatively high latitudes, or have been cultivated at northerly latitudes as annuals propagated from seed,

initiate bloom under shorter dark periods—longer day-lengths—than do populations of the same species derived from locations nearer the equator. Genetic dominance of the "short-day" plant and the subsequent appearance and selection (either natural or artificial) of "long-day" genotypes are considered to be factors of evolutionary significance in adapting the species to occupy higher latitudes.

KNOTT, D. R. The Effect on Wheat of an *Agropyron* Chromosome Carrying Rust Resistance. *Field Husbandry Department, University of Saskatchewan, Saskatoon, Sask., Canada.*

A stem and leaf rust resistant derivative produced by L. H. Shebeski from the cross, *Triticum vulgare* Vill. var. Chinese × (Chinese × *Agropyron elongatum* (Host) Beauv.), was one of the parents in the present study. Cytological studies of F_{13} plants showed that some of them had 56 chromosomes although plants with other numbers also occurred. At meiosis in most cells all of the chromosomes formed bivalents. However, configurations including one or two multivalents were not uncommon. The F_{13} plants were crossed and backcrossed repeatedly to *Triticum vulgare* var. Thatcher, using stem rust resistant progeny as the non-recurrent parents in each generation. The F_1 plants from the cross with Thatcher usually had 49 chromosomes and at meiosis multivalents were frequent although some cells had 21^{II} and 7^{I}. As the backcrossing progressed, rust resistant plants carrying a single *Agropyron* chromosome were produced. The added chromosome never appeared to pair with a wheat chromosome.

After the second and later backcrosses to Thatcher, the rust resistant plants in the progeny were selfed and homozygous resitant lines obtained. These lines proved to be either substitution lines in which a pair of *Agropyron* choromosomes had replaced a wheat pair, or addition lines in which the *Agropyron* pair had been added to the wheat complement. Monosomic analysis revealed that two of the substitution lines lacked chromosome VI. The substitution lines showed normal vigor and fertility and some yielded equally as well as Thatcher. The addition lines, however, were later and somewhat less vigorous.

Gametic transmission of the *Agropyron* chromosome was studied in plants having $21^{II} + 1^{I}$ (*Agropyron*) and also in plants having $20^{II} + 1^{I}$ (wheat) + 1^{I} (*Agropyron*). The *Agropyron* chromosome had no deleterious effect on the gametes and was transmitted with equal frequency through both eggs and the pollen. Gametes carrying 20 wheat chromosomes and 1 *Agropyron* chromosome appeared to have a slight competitive advantage over gametes carrying 21 wheat chromosomes.

KOBAYASHI, TEISAKU. Radiation-Induced Mutant Types in Sesame. *Biological Institute, Toyama University, Toyama, Japan.*

Three different forms of sesame (*Sesamum indicim* L.) cultivated in Japan, B_1D(BAN-type), WD(3BO-type), and WD_8(QAN-type), were treated with four different radiations (β-ray from P^{32}, X-ray, γ-ray from Co^{60}, and β-ray + X-ray). During a period of three years, various visible mutant types were obtained from

the treated generations and their progenies. The effects of the radiations on the external characters of sesame plant were shown by three major changes: (1) alteration of phyllotaxis, (2) development of nectary to functional flower or capsule, and (3) increase of loculus number per capsule.

B_1D(BAN-type) was more affected by the three changes due to radiations than were the other types; in consequence, a considerable number of mutant types were obtained from the B_1D(BAN-type). Among the mutants from B_1D(BAN-type), there were also types similar to the mutant types derived from WD(3BO-type) and WD_8(QAN-type). This finding suggests that B_1D(BAN-type) may be an ancestral type of the sesame cultivated in Japan, and that WD(BAN-type) and WD_8(QAN-type) may have originated from a B_1D(BAN-type)-like form.

KOCH, GERHARD. Genetics of Microcephaly in Man. *Institut für Humangenetik der Universität Münster/Westf., Germany.*

Owing to the initiative of Prof. v. Verschuer, an extensive registration of pathological traits and syndromes in man (considered to be caused by mutant genes) was started in April 1957, in a population of more than two million people, to supply data for studies in population genetics.

One of the conditions being investigated is microcephaly. From April 1957 to March 1958, 83 index cases of this disease were found through systematic search in mental hospitals. Our sample of microcephalics must be divided into two groups: hereditary and non-hereditary. Some probands died during the early period of life (i.e., within the first days or within a few months of birth). On the basis of our observations, the etiology of microcephaly cannot be explained by such circumstances as age of the mother at birth of the child, birth rank of the patient, and other secondary factors. Family studies revealed that the sibship of several index cases included additional cases affected with microcephaly. Consanguineous marriage of the parents of our probands was found in a few of the families. As yet, our material on microcephalics includes a series of three twin pairs discordant as to microcephaly. Individuals showing a minor degree of microcephaly could be considered to be heterozygotes with regard to the pathological gene. Moreover, the question of a heterogenic basis for this trait is considered. From the data of other authors as well as from our own it can be concluded, however, that the great majority of microcephalics are the result of a single-factor inheritance, namely an autosomal recessive gene.

KODANI, MASUO, and SABRO KANAI. The Karyosomal Chromosome of Man. *State University of Iowa, Iowa City, Iowa, U.S.A.*, and *Shida Hospital, Shiguokaken, Japan.*

The chromosome bearing the karyosome in man has been analyzed for the structure of the chromosome and of the karyosome as well as for the structural relationship of the two. The karyosome is found to be associated with relatively large and highly Feulgen positive "basal chromomeres" at early primary spermatocyte prophase. Under certain conditions the karyosome may be pushed away or removed com-

pletely from the "basal chromomeres" without disrupting the structure of the chromosome. Thus the karyosome does not appear to be an integral part of the linear structural continuity of the chromosome. The structure of the chromosome and of the associated karyosome will be described.

KOJIMA, KEN-ICHI, and C. CLARK COCKERHAM. Stability of Equilibrium when Selecting for Genes which Exhibit Epistasis. *North Carolina State College, Raleigh, N.C., U.S.A.*

A random mating population with two alleles at each locus, with free recombination between loci, and with interactions between non-allelic genes is considered. The necessary and sufficient conditions for mass selection to be ineffective and for the equilibrium to be stable are (1) overdominance for the marginal means of each locus when at equilibrium; (2) the matrix of which the diagonal elements are the dominance comparisons and the off-diagonal elements are twice the additive $\times$ additive comparisons is negative definite when at equilibrium. When these conditions are satisfied, fitness is at a relative maximum. Specific examples for two loci and for more loci when the effects of the loci are equal are considered.

KØLMARK, G. *See* DESERRES, F. J.

KOO, FRANCIS K. S. Induction of Somatic Mutations in a Balanced Lethal Barley Stock by X-Rays. *Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minn., U.S.A.*

Seed lots of a balanced lethal barley stock heterozygous for albino (a_n) and xantha (x_c) were exposed to 100 kv. unfiltered X-rays from a constant potential source. After treatment seeds were immediately hydrated for 45 minutes in O_2-free water through which N_2 was bubbled. Mutations of A_n to a_n or X_c to x_c, expressed respectively as albino or xantha stripes or partial stripes, on otherwise green plants were scored from the third leaves up to the eighth leaves. As used here mutation is considered in the broad sense and may be attributable either to inactivation or alteration of the dominant A_n or X_c allele, or to loss of a chromosomal segment bearing either locus. The mutation rates per plant at X-ray doses of 0, 4,000, 8,000, 12,000, 16,000, 20,000 and 24,000 r for A_n to a_n were 0, 0.080, 0.122, 0.187, 0.261, 0.272, and 0.282, and for X_c to x_c were 0, 0,027, 0.061, 0.091, 0.135, 0.152, and 0.181, respectively. For comparison, homozygous normal seeds were irradiated with 12,000 r and 24,000 r of X-rays. At these 2 dosages mutation rates for albino stripes were 0.075 and 0.115, and those for xantha stripes were 0.043 and 0.058. The mutations observed in both the control and balanced lethals may include some mutations at loci other than A_n and X_c. However, the fact that mutation rates in the balanced lethal stock were significantly higher than in the control is good evidence that mutations at the A_n and X_c loci occurred frequently. In the balanced lethal stock the mutation at A_n was more frequent than that at X_c and both

mutation rates increased linearly with dosage except that at higher doses the albino mutation approached a plateau. If chromosomal losses were frequently involved in mutations, then these results suggest that A_n may be distal to X_c.

KOSIN, IGOR L. *See* SATO, IWANE.

KOSSIKOV, K. V. The Frequency of Directed Changes of the Fermentative Properties of Yeasts as a Function of Concentration in the Medium of the Specific Substrate and of the Temperature of Cultivation. *Institute of Genetics, Academy of Sciences of the USSR, Moscow, U.S.S.R.*

It becomes ever more apparent from the experimental evidence on adaptative changes of micro-organisms to toxic substances and to new nutrition sources that these changes cannot be accounted for by spontaneous mutation and selection. The author's previous publications have shown that the specific substrate available in the medium may elicit directed changes of the fermentative properties of yeasts. It was found that the new property, viz., the capacity to produce an active ferment and to ferment the respective sugar, is steadily maintained even after the elimination of the sugar to which adaptation has been elaborated (this is replaced by another sugar) and is inherited not only through vegetative but also through sexual reproduction. In the latter segregation is noted.

The increase of sucrose concentration in the medium up to a certain limit greatly accelerates the adaptation of *Sacch. globosus* to fermentation of this sugar: the number of adapted forms increases by more than twentyfold. Not only does this evidence confirm the previous data as to the direct effect of the specific substrate on the hereditary changes of the fermentative properties but a correlation is thus found between the frequency of changes and the number of carbon molecules dissolved in the medium and reacting with the cell protoplast.

An acceleration of the process of adaptive changes of the fermentative properties of yeasts has likewise been recorded with increase in temperature of cultivation.

It may be suggested from the above evidence that the variability of the fermentative properties of micro-organisms is connected with their functional state as defined by the nutrient substrate and the cultivation temperature.

KREIG, DAVID R. Mutation Induced in Bacteriophage by Ultraviolet Light. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

Various reports have indicated that irradiation of host bacteria might be a necessary condition for ultraviolet-induced mutation of bacteriophage (Weigle, 1953; Latarjet, 1954; Tessman, 1956). Although the genetic variants that have been found in experiments involving irradiated bacteria may have been actually induced mutants, an alternative suggestion has been that they originate in some kind of radiation-induced recombination between phage and host. An investigation of induced back-

mutation in T4*r* bacteriophage was undertaken in the hope of discriminating among the various interpretations. Genetic recombination between this virulent phage and its host, *Escherichia coli*, is probably not possible. Rare r^+ phage may be selectively assayed without the complication of phenotypic mixing. Unirradiated strain B bacteria were multiply infected with ultraviolet-irradiated T4*r* phage and the progeny phage resulting from multiplicity reactivation were scored for the frequency of r^+ back-mutants. Up to fiftyfold increase of r^+ phage frequency was found after this treatment in experiments with T4*r*59. Stocks of this mutant have a very low frequency (about 10^{-8}) of spontaneous back-mutants. Parallel experiments show that the increase cannot be accounted for by favored growth of pre-existing r^+ phage. Different *r* mutants have been compared for their capacity for induced back-mutation. Initial experiments support the working hypothesis that the lower the spontaneous level of back-mutants the higher the factor of increase from radiation-induced mutation, except that genes incapable of spontaneous back-mutation also give no induced back-mutants. The question may be considered whether the role of multiplicity reactivation in this system(and possibly similar phenomena in cases involving irradiated host bacteria) is simply to permit examination of large populations after high doses. This question should be answerable by varying the conditions of multiplicity reactivation.

KROKOWSKI, E. *See* EHLING, U.

KÜHN, ALFRED, and ALBRECHT EGELHAAF. The Action of the Mutation *a* in *Ephestia kühniella* on the Formation of Kynurenin from Tryptophan. *Max-Planck-Institut für Biologie, Tübingen, Germany.*

Incubation of L-tryptophan with homogenates of larval testes, fat body, or adult ovaries of a^+/a^+ animals (*Ephestia kühniella*) results in production of kynurenin. Homogenates from *a/a* animals are ineffective. D-tryptophan and tryptamin are not oxidized. The kynurenin produced was identified by chromatographic comparison with known synthetic kynurenin, by several color reactions, and by the UV-absorption spectrum. Quantitative determinations were made by measuring the fluorescence of chromatographically separated spots. The amount of kynurenin formed *in vitro* is proportional both to the amount of tissue and to the reaction time. These results indicate that a consequence of the mutation $a^+ \rightarrow a$ is the absence or inactivating of the tryptophan-oxidizing system in the mutant tissues. To test the possibility of an inhibitory effect of the *a* homogenates, aliquots of homogenized ovaries of a^+ and *a* females were mixed and incubated with tryptophan. Kynurenin formation was not blocked; in fact, kynurenin formation was as high as if both components had been a^+. If more *a*-homogenate was added the production of kynurenin was, within limits, proportional to the amount of brei used. But the addition of heat-denaturated and resuspended *a*-homogenate did not cause an increase in kynurenin production.

It is concluded that (1) the failure of *a/a* tissues to oxidize tryptophan *in vitro* is not due to the presence of an inhibitor, and (2) it seems highly probable that at least two steps are involved in this reaction (as is already known from the

tryptophan-oxidase system of mammalian liver). That step which is not absent in a/a would be the limiting step in $a + a^+$ brei mixtures.

KUNICKI-GOLDFINGER, WLADYSLAW, TADEUSZ LACHOWICZ, and MACIEJ TABACZYNSKI. The Role of Cytoplasm in Lingering Modifications in Bacteria. *Department of Microbiology, University and Institute of Immunology and Experimental Therapy, Wroclaw, Poland.*

This study was undertaken to elucidate the mechanism of some induced, heritable variations in bacteria, observed in experimental conditions excluding the selection of spontaneous mutants.

It was ascertained that subeffective concentrations of penicillin induced in certain conditions the appearance of several variants in *Micrococcus pyogenes* v. *aureus*. These variants only rarely were penicillin-resistant and a few were penicillin-dependent. The majority of them did not differ from the mother strain in their resistance; many were slightly less resistant and some exceedingly susceptible to the antibiotic. Examination of the growth rates of these variants in various cultural conditions, with and without penicillin added, in pure as well as in mixed cultures, has shown that their appearance was due to the induction by the antibiotic rather than to the selection of spontaneous mutants. Some experiments on the kinetics of appearance of these variants suggested the alteration in cytoplasmic make-up of bacterial cells as a cause of observed changes.

This problem was further studied using *Escherichia coli*. Permanent and semi-permanent changes in streptomycin-resistance and other physiological characters were obtained as the result of some imbalance in the relative rate of formation of cytoplasmic constituents. The imbalanced growth was produced in the cells by changes in the temperature of incubation or by modifications in the composition of cultural medium.

In some defective mutants of *E. coli* the back-variation to prototroph-state induced by the presence of specific metabolites in appropriate concentrations, when some synthetic reactions in the cells were impeded relative to others.

Although the biochemical mechanism of all these changes was not studied it would seem reasonable, on the basis of results obtained, to consider this process as a kind of heritable cytoplasmic alteration ("Dauermodification").

KUPZOW, A. J. A Parallelism in Variablity of Plant Species with Certain Common Characters. *Moscow, U.S.S.R.*

Vavilov's law of the homologous series has an enormous value for genetics and plant breeding. This law establishes a parallelism in the evolution of constructively similar genotypes. But its author restricts this law considerably when he writes that the homologous series of variation are characteristic for taxonomically near groups of species, in spite of the homologous series in the variability of root-crops and different trees, which were known to him and were cited in his papers. This nonconformity of the law formulation with the facts on which is is based was not noticed by Roemer either. In the meantime the extension of Vavilov's

law beyond the limits of taxonomically near groups of species is necessary according to its essential spirit.

It is now evident that all genetic similarity is the cause of homologous series in variability. This similarity can be in characters of taxonomical value, and therefore we have homologous series in species of one genus or in species of one family. But there are similar evotypes of different species from remote genera and families, and we have also for this reason homologous series in groups of analogous ecotypes of different species. Some characters without taxonomical and ecological value can also be similar in remote species. Therefore the root crops have the homologous series in the form of the roots, the different trees in the form of their tops, the Mexican *Cactaceaes* and South African *Euphorbiaceans* in the form of their succulent stems, and species with certain basic pigments in the colour of flower, fruit, seed and other organs.

The first cause of this phenomenon is the nomogenic similar mutations of genotypes with common elements of structure. Our taxonomy reflects only the similarity in certain parts of genotypes (the genes of morphological characters of taxonomical value), while their other parts can be very different in one taxonomical group. And vice versa, certain genotypes in different distant taxonomical groups can be very similar in their separate parts (the genes of physiological value or of any morphological characters without taxonomical value).

The second cause of the cited parallelism in variability is evidently the elimination of abortive genetic complexes rising as a result of mutations by selection.

Thus the parallelism in variability probably leads to the logical necessity of accepting the nomogenic mutations as an essential factor of the evolution. These mutations are controlled by natural and artificial selection in the case of adaptive or useful characters, but they are beyond this selective control in the case of ecologically and economically indifferent characters. Nevertheless these nomogenic mutations do not diminish the leading role of selection in the evolution, but emphasize it still more, since the selection of original forms predetermines their mutations and therefore directs their future evolution even in characters indifferent for selection.

KUSHNER, DONN J. Development and Properties of Alkali-Resistant Strains of *Bacillus cereus*. *Forest Insect Laboratory, Science Service, Canada Department of Agriculture, Sault Ste. Marie, Ont., Canada.*

Bacillus cereus, a pathogen of the larch sawfly, *Pristiphora erichsonii* (Htg.), grows in the midgut of the larva and is thought to attack it by producing a toxic phospholipase there. The midgut pH range (7.2–8.5) of larch sawfly larvae is one in which the bacteria can multiply rapidly, but in a number of lepidopterous larvae, the midgut pH is too high (9.5–10.5) for normal *B. cereus* to grow. It was thought that if a strain of *B. cereus* could be induced to grow at such high pH values it might prove pathogenic for Lepidoptera.

By extended serial cultivation in media of progressively higher pH, a strain of *B. cereus* has been developed that can grow at pH 10.5. These organisms, and others trained to grow at intermediate pH values, are being maintained on buffered alkaline agar. Their alkali resistance is not lost upon several transfers on neutral medium.

Alkali resistance appears to develop in a stepwise manner through exposure to alkaline medium and is not due to selection of mutants originally present. Inoculation of even large numbers of the sensitive organism into highly alkaline medium does not result in growth. The resistant organisms retain their rod shape but are shorter and thinner than the original strain. Resistant organisms growing on alkaline medium lower the pH of the medium. The fall in pH accompanies but does not precede growth. A discussion of the possible mechanism of resistance as well as of the mode of development of resistance will be given.

KUSHNER, H. F. Inheritance of Changes in Feathering Pigmentation in Fowls (hens) Subjected to Foreign Blood Transfusion. *Institute of Genetics, Academy of Sciences of the USSR, Moscow, U.S.S.R.*

The pattern of inheritance of pigmented feathering observed in White Leghorns following the transfusion to the latter of blood from New Hampshires and gray semi-wild ducks has been studied.

Hens of the second generation with pigmentary spots in their feathering, when mating with cocks (also of the second generation) having the same partly pigmented feathering from the experiment in which the parental pairs were not subjected to foreign blood transfusion, produce a third generation of chicks 26% with the parental type of feathering and 34% with entirely pigmented gray-striped feathering (barred).

In the parental pairs of the second generation, the parents themselves were pure white notwithstanding the fact that their ancestors underwent blood transfusion. Where the parents themselves were not subjected to any more blood transfusion, 17.3% of the offspring had partially pigmented feathering and 1.9% were pigmented all over.

Other similar experiments suggest that blood transfusion in hens—even when it does not seem to affect the colour of feathering of the first generations—frequently affects their heredity since in subsequent generations, repeated foreign blood transfusion being excluded, chicks appear with pigmented feathering.

The hereditary nature of the observed changes in birds' feathering is also confirmed by the following experiments.

When mating F_2 gray-striped hens with similar cocks of the second generation (without subjecting them to the foreign blood transfusion), 93% of the resulting chicks had fully gray-striped pigmentation. (barred). When mating experimental cocks of the second generation, themselves partly pigmented, with the control pure-bred White Leghorn hens, ancestors thereof and themselves not being subjected to blood transfusion, 23.5% of offsprings had partly pigmented feathering. When mating such control Leghorns with an experimental gray-striped cock of the second generation 64% of offsprings had pigmentary spots in their feathering.

In a specially conducted control test White Leghorns were subjected to blood transfusion from the hens of the same strain; in the first generation so far studied no deviations from the standard feathering of this strain have been observed.

The physiological processes responsible for the observed hereditary variations in the pigmentation of feathering (as well as in that of the beak and legs) are still obscure, but further studies are being conducted.

LaChance, Leo E. The Role of Chelation in the Production of X-Ray Induced Dominant Lethals in *Habrobracon. Genetics Department, N.C. State College, Raleigh, North Carolina, U.S.A.*

Four groups of virgin *Habrobracon* wasps, comprising (1) controls, (2) EDTA-fed, (3) X-irradiated, and (4) EDTA-fed and X-irradiated, were compared for egg production and hatchability subsequent to treatment. The percentage of embryonic dominant lethals was calculated by the comparison of hatchability of the eggs from females given identical treatments, some mated and some left unmated for the parthenogenetic production of haploid males.

Data were collected throughout the reproductive life of the females. Those embryos which were derived from eggs in different stages of meiosis and oogonial mitosis at the time of treatment were identifiable on the basis of ovariole morphology.

Females were allowed only a single meal of the chelating agent, 0.1 M EDTA as the disodium salt. In those groups which were fed the chelator and then irradiated, the dominant lethality was much greater than it was in either the irradiated or the EDTA fed groups. It was also greater than could be expected if the two agents were independent in action or merely additive. Thus the action of the two agents is believed to be synergistic.

The role of EDTA in the enhancement of induced genetic lethals can be attributed perhaps to one or more of four pathways: (1) the chelation of metal ions at the site of ionic breaks, which would prevent the normal restitution of the chromosomes; (2) enhancement of radiation damage via the inhibition of enzymes necessary to normal restitution, since many enzymes important in biosynthesis are dependent on metal ions for proper activity; (3) inhibition of the Fe dependent enzymes peroxidase and catalase which are responsible for the biological breakdown of peroxides formed in tissue irradiated in the presence of oxygen. (4) interference with normal restitution of chromosome breaks due to altered viscosity of the nucleoplasm.

(This investigation was supported by a predoctoral research fellowship (AF–6540) from the National Institutes of Health, U. S. Public Health Service. Appreciation is expressed to the Dow Chemical Company for providing the chelating agent.)

Lachowicz, Tadeusz. *See* Kunicki-Goldfinger, Wladyslaw.

La Cour, L. F., and S. R. Pelc. The Incorporation of Tritiated-Thymidine in the Nuclei of Roots of *Vicia faba. John Innes Horticultural Institution and Biophysics Dept., King's College, London, England.*

The incorporation of tritiated-thymidine in the DNA of nuclei in roots of seedlings of *Vicia faba* has been studied by the autoradiograph method. In meristematic cells the labelled compound is incorporated only during a part of interphase. Autoradiographs of mitotic figures following growth with and without colchicine indicate that the hypothesis of chromosome duplication proposed by Taylor *et al.* (1957) may need some modification. A proportion of resting nuclei in the zone of cell elongation gives more intense autoradiographs than do nuclei in the

meristem of the root tip. At first this more intense labelling is observed in nuclei immediately adjacent to the 2–3 mm. of meristem but with time it extends further into the zone of root elongation.

Photometrical measurement of Feulgen stain in resting nuclei adjacent to the meristem has shown that a large proportion of them contain a 4C (prophase) amount of DNA. Thus the intensity of labelling in these nuclei cannot be attributed either to polyteny or to polyploidy. It is suggested that unlabelled thymidine is replaced by labelled thymidine in unlabelled strands in the chromosomes at the time these cells cease to be meristematic.

LACY, ANN MATTHEWS, and DAVID M. BONNER. Complementarity between Alleles at the Td Locus in *Neurospora crassa*. *Department of Microbiology, Yale University, New Haven, Conn., U.S.A.*

The genetic locus which controls the formation of the enzyme tryptophan synthetase in *N. crassa* has been termed the Td locus. This enzyme, which carries out the final step in tryptophan synthesis, is measured by its ability to catalyze the condensation of indole and serine to form tryptophan. In the past few years, a number of independently occurring mutants defective at this locus have been isolated. All of these mutants are phenotypically similar in that they do not form an amount of tryptophan synthetase detectable by the enzyme assay. Some of these mutants have been shown to differ from each other in terms of temperature sensitivity, specificity of suppressor genes, formation of immunologically cross-reacting material, and indole accumulation, suggesting that the effect on tryptophan synthetase production is not the same in each instance. A functional difference between the isolates is also suggested by the formation of occasional pseudo-wild type progeny (intra-nuclear complementation) from crosses between certain of these mutants.

These observations made it desirable to study the possibility of heterocaryon formation between Td alleles. Preliminary tests gave no indication of complementarity. However, various mutants were back-crossed to a heterocaryon positive (het^+) wild type organism and more nearly isogenic mutant strains isolated. Combinations of these isolates were tested and some were found to form tryptophan independent heterocaryons, although the time required for their formation was considerably longer than that for heterocaryon formation between non-allelic mutants.

The results of these studies indicate that at least three groups of mutants can be determined on the basis of complementarity. The characteristics of the tryptophan synthetase formed by various heterocaryons is currently under investigation and will be discussed.

LAGERVALL, MATS. The Correlation among Individuals Caused by Dominance. *Department of Animal Husbandry, Iowa State College, Ames, Iowa, U.S.A.*

The "coefficient of relationship" (Wright, Genet. *6*: 111–143) and the "coefficient de parenté" (Malécot, Les mathématiques de l'hérédité, Paris, 1948) express in slightly different ways the relationship between two individuals on a purely additive basis. Both are functions of Wright's inbreeding coefficient, F. In this study an

attempt was made to evaluate the corresponding relationship that is caused by dominance.

Suppose the individuals X and Y possess the genotypes ab and cd, respectively. The correlation between X and Y due to dominance can then be expressed as P (ab = cd). For calculating purposes it can be written as P (a = c) P (b = d/a = c) + P (a = d) P (b = c/a = d) − P (a = b = c = d).

For regular inbreeding systems P (ab = cd) can be evaluated with the help of mating matrices (for the algebra, see, e.g., Kempthorne, An Introduction to Genetic Statistics, New York, 1957) and expressed as a function of F. However, even for the full sib mating the function turns out to be rather complicated. In irregular inbreeding systems the event (a = c) is often independent of the event (b = d) as well as the event (a = d) of the event (b = c). In such cases the above-mentioned formula can be simplified and P (ab = cd) can be written as a function of F in the following way:

$$F_{A\times C}\, F_{B\times D} + F_{A\times D}\, F_{B\times C} - F_{XP}\, F_{YP},$$

where $F_{A\times C}$ = the inbreeding coefficient of the offspring that would theoretically result from a mating between X's sire (A) and Y's sire (C); B = X's dam; D = Y's dam; F_{XP} = the inbreeding coefficient of X with respect to P, an ancestor common to A, B, C, and D; etc.

The probability dealt with in this paper was denoted u_{XY} by Kempthorne (1957) and as such included in the general formula for cov (X, Y). Besides the value this coefficient of relationship caused by dominance has in itself, its estimation is thus necessary for the calculation of cov (X, Y). (This work was done during the tenure of an F. A. O. André Mayer Research Fellowship. Financial aid was also given by Helge Ax:son Johnsons Stiftelse, Sweden, and by Skandinaviska Bankens Stipendiefond av 1931, Sweden.)

LANDAUER, WALTER. On Phenocopies, Their Developmental Physiology and Genetic Meaning. *University of Connecticut, Storrs, Conn., U.S.A.*

A review will be given of recent studies on the chemical production of phenocopies in *Drosophila* and chicken embryos. The first part of the review will present the general conclusions to which this work has led in regard to the external and internal factors which play a role in the origin of phenocopies. These conclusions will then be discussed with reference to their meaning for developmental physiology and genetic theory. It will be maintained that it is unnecessary to assume specific isoalleles and specific modifier systems for the interpretation of the known facts. On the contrary, it will be proposed that phenocopies arise as reactions of particular genotypes to external conditions with which the homeostatic forces of the developing organisms are unable to cope.

LASKOWSKI, WOLGANG, and WERNER STEIN. The Effects of X-Rays, UV, and other Inactivating Agents on the Budding Ability of Saccharomyces Strains of Different Ploidy. *Max-Planck-Institut f.vergl. Erbbiologie and I.Physikalisches Institut, Free University, Berlin-Dahlem, Germany.*

The microscopic pattern of rest-budding in X-ray, UV, or peroxide-treated yeast cells was studied in haploid and diploid strains of Saccharomyces.

UV-irradiation resulted in all investigated strains in a very similar pattern, i.e., by a given percentage of macroscopic survivals approximately the same final frequencies of more than one and also of more than three cells in a clone. It is possible to explain these results by a non-strain-depending dose reduction factor of 2.2 : 1 for the frequency of more than one cell, and 1.2 : 1 for the frequency of more than three cells, both related to the commonly used macroscopic survival frequency curve. The dose reduction by photo-reactivation was also approximately 2.2 : 1 and caused essentially a corresponding change of the pattern.

After treatment with organic peroxides the pattern in haploid strains was very similar to that after UV treatment although there was no photo-reactivation. In diploid strains there is a characteristically different pattern with almost none rest-budding. It seems remarkable that this basic change of the pattern occurred even if an endomitotically originated diploid strain was tested. This strange phenomenon demands a specific genetic interpretation.

For X-ray treated strains there could also be observed a dose reduction principle for the criteria $>$ 1 cell, $>$ 3 cells, and macroscopically visible clones. For a haploid strain these dose relations were about 30 : 1.33 : 1, and for a diploid strain about 10 : 1.65 : 1. In one respect the X-ray induced pattern was different compared with the pattern after UV treatment: the number of single cells after more than 48 hours incubation was—at least within a certain dose range—significantly smaller than after UV treatment. In reasonable agreement with this fact there was nearly no retardation of the first budding step. This holds for every strain and every dose tested. In contrast with the X-ray experiments a retardation of the first budding step was observed after UV and peroxide treatment in all strains tested. The criteria used in these experiments offer new aspects for a discussion of inactivating mechanisms.

LAUPRECHT, EDWIN, and EDWARD WALTER. On the Valuation of Methods to Form Certain Gene Combinations by Breeding. *Max-Planck-Institut für Tierzucht und Tierernährung, Abteilung für Tierzüchtung und Haustiergenetik, Niedersachsen, Germany.*

There are different methods of forming a certain gene combination $A_1A_1\ A_2A_2 \ldots A_nA_n$ by breeding when starting with types such as $A_1A_1 \ldots A_mA_ma_{m+1}a_{m+1} \ldots a_na_n$ and $a_1a_1 \ldots a_ma_mA_{m+1}A_{m+1} \ldots A_nA_n$ where the final aim can be approached slowly by alternately crossing and selecting. After each crossing the resulting combinations can be classified in states by the extent of their approach to the desired gene combination. The breeding method to be applied here can be represented by a stochastic matrix (p_{ik}) similar to the representation of a regular system of inbreeding by a generation matrix; p_{ik} is the probability for the transition from the i^{th} to the k^{th} state in a time unit. If no step in the breeding process will result in a decline, then $p_{ik} = 0$ for $k < i$. In this case the eigenvalues of the stochastic matrix are given by p_{ii}. The value of a breeding method depends on the average number of time units or the average number of individuals necessary to establish a desired gene combination.

Another way of evaluating a breeding method is based on the number of time units necessary to establish with a given probability α the desired gene combination. When α is large it is sufficient to use the largest non-unit eigenvalue of the matrix

as a measure. These methods of valuation can be applied in self-fertilised as well as in cross-fertilised populations.

LAVEN, H. Cytoplasmic Inheritance and Speciation in Mosquitoes. *Max-Planck-Institut für Biologie, Tübingen, Germany.*

The family of mosquitoes (Diptera:Culicidae) is rather uniform in morphology. Studies on the bionomics of anopheline mosquitoes have revealed that in several instances the morphologically defined species are, in fact, complexes of sibling species. Crossing experiments among populations of the common mosquito *Culex pipiens* from Europe, Africa and North America have shown that this "species" also contains an undefined number of sibling species. Full isolation or unilateral isolation of the sibling species in this group comes about by cytoplasmic inheritance. Factors, as yet unknown, which are independent of chromosomal genes determine the viability of hybrid embryos. The cytoplasmic agents are characteristic for every "species" in the complex and they are transmitted by way of the egg cytoplasm as well as by the sperm. Some observations in other genera of the culicines give support to the idea that this long unknown isolation mechanism might be effective generally in the family of mosquitoes for speciation and evolution.

LAWLER, SYLVIA D. Some Observations on the Gm Serum Protein Groups in Man. *Galton Laboratory, University College, London, England.*

The Gm serum groups in man were discovered by Grubb (Acta path. microbiol. scand. *39*:195). There are two phenotypes, Gm(a+) and Gm(a—). The groups are determined by a serological system involving the following carefully selected constituents: human Rhesus positive red cells strongly sensitized with an "incomplete" anti-D serum + serum from a patient with rheumatoid arthritis → agglutination. The agglutination is inhibited if serum from a Gm(a+) individual is added to the system; it is not inhibited, or inhibited but weakly, by the serum of a Gm(a—) individual.

A study has been made of the serology and genetics of these groups. Some results of population and family studies in Great Britain and Ferrara (Italy) will be given.

LEBEDEV, M. M. Influence Exercised on Characteristics of Offspring by Premating Treatment of Animals and Fertilization Conditions. *Pushkino Scientific Research Laboratory for Stock Breeding, Moscow, U.S.S.R.*

Charles Darwin used to say on the basis of his observations that there was hardly a more amazing thing in nature than the sensitivity of sex cells to external influences. The intensive assimilation activity on which this phenomenon is based can be clearly observed in experiments carried out by means of tracer atoms. Radioactive P^{32} and Ca^{45} introduced into the food or directly into the blood stream of the animals, rapidly accumulate in the spermatozoa of cocks, rabbits and rams, as well as in chicken eggs. Experiments made with the intervarietal transplantation

of gonads in animals and with blood transfusion in chickens prove the possibility of changing the properties of offspring by establishing new conditions for the development of gametes.

The characteristics and viability of offspring largely depend on the differentiation of the gametes taking part in the fertilization process, on the nature of the food received by the animals and their maintenance previous to mating, their age and condition at the moment of mating.

Fertilization is a complex process of mutual assimilation by the merging sex cells. The ultimate maturing of the ova and their fertilization take place during their contact with more or less numerous spermatozoa. A particular feature of the fertilization process in all farm animals consists in the mass penetration of spermatozoa into the transparent membrane of the ovum. Not the head of the spermatozoon alone, but the entire spermatozoon including its tail penetrate into the plasm of the ovum.

In a number of cases several spermatozoa penetrate into the plasm of the ovum simultaneously, leading to the formation of three or more pronuclei. In chickens the mass penetration of dozens and even hundreds of spermatozoa into an ovum is the rule. The spermatozoa often penetrate both into the ovum and into the zygotes in the process of division.

Numerous experiments carried out with rabbits, pigs and chickens show that in these animals and birds fertilization is of a selective nature.

A dependence has been established between the number of spermatozoa which penetrate into the ova and the zygotes, the fertilization rate and the development of the embryo. Heterospermatic insemination of females with the semen of different males as well as an additional insemination of females after the termination of the basic fertilization enhances as a rule the viability of the offspring. On the basis of experiments carried out with chickens, there is reason to believe that heterospermatic insemination can, in certain cases, influence the heredity of the offspring.

All these experimental studies are graphic proof that by changing the conditions of fertilization man can exercise a considerable influence on the characteristics of the offspring. An example of the active interference into the fertilization process is the so-called double mating method of pigs evolved by the Pushkino Laboratory of Stockbreeding.

The application of this method to hundreds and thousands of pigs in large herds has contributed to a considerable reduction in the barrenness of sows, to the heightening of their fertility, and to improved characteristics of their offspring.

A tremendous influence on the developing foetus is exercised by the maternal organism, a fact which has been proved by numerous observations of reciprocal crossing of animals belonging to different varieties and species, as well as by experiments on the vegetative hybridization of animals.

LEDERBERG, ESTHER M. Fine Structure of the Gal Loci in *Escherichia coli* K-12.
Genetics Department, University of Wisconsin, Madison, Wis., U.S.A.

A distinctive recon (one of a group of closely linked loci separable by recombination) has been assigned to each of ten independently occurring Gal — (galactose non-fermenting) mutants. All the mutants were obtained after UV irradiation except Gal_3 which was spontaneous. Allelism was tested by large-

scale matings and by transduction analysis. Prophase-linked transduction via HFT λ, where practically every phage particle may result in a transductional event, produced heterogenotic clones when donor and recipient bore distinctive recons. The heterogenotes were unstable, segregating the two input and two cross-over classes (Morse, Lederberg and Lederberg, 1956). Two cistrons (cis-trans position effect groups) had been demonstrated: with members of the same cistron trans heterogenotes are phenotypically mutant. Galactokinase was missing in mutants of one cistron (Gal 2,8) while UDP transferase activity was absent in the other (Gal 1, 4, 6, 7), a defect corresponding to human congenital galactosemia, (Kalckar, Kurahashi, 1957). Gal_3^- and Gal_9^- are genetically cistronic with both of the foregoing groups; they have not yet been successfully analysed for their enzymatic defect. Among two hundred new mutants, no recons identical with the first ten have recurred. Most have been assigned to either of the first two cistronic groups, but a small number which would represent a fourth genetic group have given only normal galactose-positive heterogenotes with every standard Gal$^-$ tested. A few mutants were not transformable by λ but were found to carry other modifiers including that of hexose metabolism which obscure their relationship to the Gal$^-$ group. An attempt was made to map the Gal markers in linear sequence by their relationship to the Lp locus. Intercrosses of Hfr $M^-Gal_x^- \times F^-Gal_y^-$ were made on a medium which selected efficiently for $M^+Gal_x^+Gal_y^+$ recombinants, and these were then scored for the segregation of an Lp marker. However, the linkage of Gal-Lp proved to be not close enough to compensate for the perturbations of segmental loss from the Hfr parent, and the data were indecisive.

Lee, Woong-Jik. Physiological Effect of Gamma-Radiation on Meiosis in Rye. *Department of Biology, College of Education, Seoul National University, Seoul, Korea.*

During the work concerning the effect of gamma-radiation on meiosis in rye, in 1956, a marked physiological effect was observed. A vernalized Korean rye containing 14 ordinary chromosomes was irradiated with Co^{60} in dose of 150 r (7 r per minute). The temperature during irradiation was maintained between 21° and 22°C. In control or before irradiation, meiosis was quite normal except for unpaired chromosomes and chromosome bridges in a few cells. Chromosomes showed uniform stain capacity.

Immediately after irradiation, inhibition in meiosis was noticed. At the first metaphase, many bivalents formed rings and pronounced nucleic acid starvation was found in most chromosomes. Heterochromatic regions were found to be spherical hollows or holes in a majority of chromosomes. In extreme cases undercharging in nucleic acid was so marked that coiled spirals were visible.

Six hours after irradiation, gradual recovery was noticed although nuclear development was still inactive. Nucleic acid starvation was not so serious as in the previous stage, but surface stickiness and fusion of chromosomes were very apparent. In 12 hours, recovery was more marked. Sticky bridges, dicentric chromosome bridges accompanied with acentric fragments, and lagged fragments were found in many first anaphases. In 24 hours, suitable stage for analysis had not been obtained, presumably because of an upset in timing by irradiation.

It is clear that nucleic acid starvation in chromosomes immediately after

irradiation was followed by surface stickiness and fusion of chromosomes due to overcharged nucleic acid. Then it proceeded toward chromosome breakages.

(I wish to thank Professor P. T. Thomas for his advice. An award of a grant by the British Council is gratefully acknowledged.)

LEGATES, J. E., B. R. FARTHING, and C. C. COCKERHAM. Selection for Growth and Maternal Performance in Mice. *North Carolina State College, State College Station, Raleigh, N.C., U.S.A.*

Selection for individual 6-week weight and 12-day weight of a litter of 6 mice has continued for 9 generations in a population arising from a cross of 4 inbred lines (A, BALB/c, AKR, DBA/2) obtained from the Roscoe B. Jackson Memorial Laboratory. Altogether 4 selection lines, for low and high 6-week weight and for low and high 12-day weight, are maintained as populations of 10 males each mated to 2 females. A control population consisting of 15 males each mated to 2 females is also maintained.

The first 9 generations of intra-litter selection for 6-week weight has resulted in a divergence equal to 24.1% of the performance in the base population (mean, 18.6 gm.). Selection for 12-day litter weight has resulted in a divergence equal to 13.0% of the performance of the base population (mean, 39.9 gm.). There have also been correlated responses for each of the above traits equivalent to 16.7% of the mean for 6-week weight and 10.5% of the mean for 12-day weight.

Our control has followed the high line for 12-day weight quite closely, but it has remained more nearly equidistant between the low and high 6-week weight line. The control was reconstituted and its performance at the time of the ninth generation of selection was slightly superior to the contemporary performance of the control line.

A cross nursing study also has been employed to assess the relative contribution of prenatal and postnatal factors on body weight at 5, 12, and 21 days and 6 weeks. In our material postnatal influences were found to be most important in determining weight through weaning (21 days). Postnatal effects accounted for 71.5% of the variance in the 12-day weight of litters of 6 mice, which is the measure of performance used in selection.

LEJEUNE, JERÔME, RAYMOND TURPIN, and MARIE ODILE RETHORE. Sur les variations de la masculinité dans la descendance de parents irradiés. *Faculté de Médecine, Paris, France.*

Ainsi que l'ont montré de précédentes enquêtes, la masculinité à la naissance (nombre relatif de garçons) diminue dans la descendance des femmes dont les ovaires ont été irradiés. L'irradiation des gonades paternelles semble influer elle aussi sur la masculinité de la descendance, mais le sens de cette variation pourrait dépendre des modalités d'application du rayonnement. La présentation et l'analyse de données nouvelles autorisent une discussion sur l'existence et la signification de ces phénomènes.

LERMAN, L. S. Estimation of the Critical DNA Segment Required for Genetic Transformation. *Department of Biophysics, University of Colorado Medical Center, Denver, Col., U.S.A.*

The kinetics of the loss of transforming activity of pneumococcal deoxyribonucleic acid indicate that each particle is inactivated by the introduction of a single molecular defect. Since the extent to which the inactive DNA is incorporated by competent cells is only slightly affected, it is likely that the transformation failure lies in genetic processes. Where the rate of accumulation of molecular alterations can be determined independently, the length of the active DNA segment which is vulnerable to a single attack can be calculated.

Results of these calculations by several independent methods indicate that the critical segment contains roughly three thousand nucleotides, and constitutes, therefore, only a fraction of the length of an intact DNA molecule, which is of the order of twenty thousand nucleotides. Neither the estimate based on the rates of hydrolysis and inactivation by deoxyribonuclease nor that based on ultraviolet inactivation requires a direct measurement or assumption regarding molecular weight.

The lengths of the critical segments have been determined for the unlinked transformations to resistance to streptomycin, novobiocin, and optochin. These are found to be identical within experimental error.

LERNER, I. MICHAEL, EVERETT R. DEMPSTER, and NOBUO INOUYE. Preliminary Report on X-ray Induction of Variability in Polygenic Traits of Chickens. *University of California, Berkeley, Calif., U.S.A.*

A flock of White Leghorns has been under continuous selection for egg production since 1933. The mean number of eggs laid annually per pullet housed is currently about one hundred eggs higher than in the foundation stock. It is, however, not entirely clear whether selection gains have now ceased although it appears highly likely that they have decelerated. An experiment intended to determine whether genetic variation in egg-production characters can be induced by X-rays and subsequently utilized to obtain further genetic progress was initiated in 1952. The first phase of this study consisted of propagation without artificial selection of a population (the *PX* line) extracted from this flock (designated *P*) in which the semen used to fertilize the dams was exposed to X-ray doses of either 1,000 or 1,500 r. A mating system approaching panmixia was used, and a control population (*PC*) reproduced in a manner identical to that for the *PX* line but without irradiation. After cumulative doses of 2,000, 3,000 and 5,500 r were applied, small contemporary samples of pullets from the *P*, *PC* and *PX* populations were produced without irradiation and data on a number of their production traits gathered. In addition, several tests on hatchability without irradiation of the *PC* and *PX* lines were carried out. Embryo viability seems definitely affected by exposure of semen of grandsires and more remote ancestors to X-rays. Thus the hatchability of the *PX* line is roughly half that of the *PC*, while crosses between them are intermediate. Probably both the means and variances of sexual maturity, egg number and egg weight have been affected. However, the answer to whether the induced variation can contribute to selection progress, will have to wait upon the outcome of the second stage of the experiment in which selection for egg production will be resumed in both the *PX* and *PC* lines.

LERNER, I. MICHAEL. *See* DEMPSTER, EVERETT R., *et al.*; LOWRY, DOROTHY C.

LESINS, KARLIS. Progenies from a Cross *Medicago dzhawakhetica* × *M. sativa*. *Department of Plant Science, University of Alberta, Edmonton, Alta., Canada.*

The original hybrid (*Medicago dzhawakhetica* × *M. sativa*) was a hexaploid ($2n = 48$) though both its parents were tetraploids ($2n = 32$). Morphological as well as physiological characteristics indicated that four genomes of *M. dzhawakhetica* were united with two genomes of alfalfa, *M. sativa,* the genomic constitution being DDDDSS. On backcrossing to tetraploid alfalfa a progeny of constitution DDSSS ($2n = 40$) was obtained; on repeated backcrossing this in turn gave DSSS $\frac{S}{2}$ ($2n = 36$). Another series of progenies was initiated by crossing the hybrid with a hexaploid alfalfa plant, DDSSSS being produced. Backcrossing this to hexaploid and tetraploid alfalfas gave progenies of constitution DSSSSS ($2n = 48$) and DSSSS ($2n = 40$) respectively.

Physiological traits, such as requirement of cold pretreatment for flower induction (a characteristic of *M. dzhawakhetica*), varied greatly with genomic constitution, though some variation was observed between plants of the same progeny. Thus, DDSSSS plants flowered freely, but some DDSSS plants rather sparsely, without pretreatment in cold.

Variation in morphological traits had the same trend. The large, articulate pod hairs characteristic of *M. dzhawakhetica*, dominant also in the hybrid, were represented only by rudiments on pods of DDSSSS, while on pods of DDSSS they were better developed; in some plants more so than in others.

The mean value of chromosome number of different progenies was lower than theoretically expected. There are several possibilities of genetic changes taking place during future generations: one, chromosomes of odd genomes may be lost without trace from the germ track; second, some extra chromosomes of *M. dzhawakhetica* or *M. sativa* may be incorporated in the genetical make-up; and further, some genetic exchange between chromosomes of both species may occur. Any hereditary change of economic value, which was the original objective of the cross, will be sought in coming generations.

LEUPOLD, URS. Studies on Allelism in *Schizosaccharomyces pombe*. *Institute of General Botany, University of Zurich, Zurich, Switzerland.*

A quantitative analysis of the meiotic formation of rare prototrophs in intragroup crosses between adenine-requiring mutants of two different groups (ad_2: 3 mutants accumulating hypoxanthine; ad_7: 11 mutants forming red pigment) has revealed a rough additiveness of the prototroph frequencies measured. This, together with the observation that any diploid clone combining two mutant genes of the same group is mutant in phenotype, suggests that these frequencies (which range from 3 to 750 prototrophs/10^6 ascospores) reflect frequencies of crossing-over recombining different mutable sites (ad_2: 3; ad_7: 9) within small chromosome sections which as a whole are acting as functional units. This interpretation may also apply to the mating type region, since crosses between heterothallic clones of opposite mating type are found to yield rare homothallic segregants (0.3%) which regularly

recombine for neighbouring markers, suggesting that crossing-over between two different sites of the mating type locus is involved.

However, efforts to demonstrate the simultaneous formation, to be expected on the crossing-over hypothesis, of two complementary recombinants per recombination event, have not been successful so far. For the ad_7-region, an analysis of half-tetrads (i.e., of the haploid progeny of the rare prototrophic cells which are formed by mitotic recombination, at a rate of 0.3–3.5 reversions/10^6 cell divisions, within diploids combining mutant genes of independent origin) has failed, in 17 prototrophs analysed, to yield the reciprocal double mutant expected. Similarly, a tetrad analysis of the meiotic formation of rare homothallic recombinants, though confirming the intimate connection with crossing-over between neighbouring markers, has failed to demonstrate the simultaneous formation of a complementary recombinant recognizable by its mating behaviour; but it still remains to be determined whether this lack of reciprocity in phenotypic segregation (2 heterothallic + : 1 heterothallic — : 1 homothallic in 10 tetrads analysed) reflects a non-reciprocal segregation on the genetic level as well.

LEVINE, LOUIS, and JOHN A. BEARDMORE. Environment, Adaptation and Balanced Polymorphism in *Drosophila pseudoobscura*. *Biology Department, City College of New York* and *Department of Zoology, Columbia University, New York, N.Y., U.S.A.*

(i) The balanced polymorphism found in the third chromosome of *Drosophila pseudoobscura* is reflected in the equilibrium frequencies of the gene arrangements involved. These equilibrium frequencies are completely predictable (given flies from the same geographic population) in laboratory populations kept at 25° C, no matter what the initial frequencies may be. At 16° C, no change occurs, the adaptive values of all genotypes being equal. At intermediate temperatures it would seem reasonable to expect intermediate frequencies of gene arrangements. However, in two intermediate environments, replicated populations gave results which were extreme in terms of gene-arrangement frequencies at equilibrium and it is possible that these equilibria are stable even in new environments.

(ii) Crosses involving different gene arrangements between flies from different geographic populations have been shown often to be indeterminate in respect to an equilibrium. This means that it should be possible to demonstrate differences in heterosis in combinations of chromosomes derived from different laboratory populations both within and between environments as compared with crosses derived from a single population. Work testing this theory is in progress and will be reported on.

LEVINE, MYRON, and ROY CURTISS. The Induction of Aberrant Recombination Frequencies with Phage P22 of *Salmonella typhimurium*. *Biology Department, Brookhaven National Laboratory, Upton, N.Y., U.S.A.*

The clear-plaque mutants of the temperate phage P22 of *Salmonella typhimurium* affect the ability of the phage to become prophage. These mutants can be classified into three distinct but closely linked functional units or cistrons on the basis of

their physiological interactions and their behavior in genetic crosses. The different mutants of a cistron have been mapped in a linear order (pseudoalleles). Crosses between many combinations of two clear-plaque mutants have been carried out. The rare wild-type temperate progeny phages arising by recombination between the closely linked mutant loci were examined for additional recombinations between the clear-plaque region and markers on both sides of the clear-plaque region. The four possible recombinant classes from such a cross are: a single recombinant class (the recombination taking place between the mutant loci), two double recombinant classes (the second recombination taking place to one or the other side of the clear-plaque region) and a triple recombinant class (the additional recombinations taking place on both sides of the clear-plaque region). Crosses with most of these mutants gave the expected frequencies of the four classes. Aberrant results were obtained in a few crosses between particular mutants. In these crosses one of the classes of double recombinants was larger than the single class. This extreme negative interference can be induced by two different treatments in crosses in which it does *not* normally occur. (1) Ultraviolet treatment of phage before infection causes the double recombinant class to exceed the single class. (2) Exposure of phage-infected bacteria early in the latent period to the antibiotic, chloromycetin, also produces this aberrancy. A number of possible explanations for these results will be discussed. (Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.)

LEVINE, R. P. *See* EBERSOLD, W. T.

LEWIS, HELEN S. *See* LEWIS, HERMAN.

LEWIS, HERMAN W., and HELEN S. LEWIS. Genetic Control of Tyrosinase Systems in *Drosophila melanogaster*. *Massachusetts Institute of Technology, Cambridge, Mass., U.S.A.*

Genetic control of the tyrosinase system (enzyme, activator and inhibitor) of *Drosophila melanogaster* has been investigated with special reference to the action of the temperature mutant sable (s) and the interacting locus, suppressor of sable (su—s). Enzyme systems are prepared for analysis by homogenization of flies in phosphate buffer (pH 7.5) followed by extraction of lipids with chloroform. All operations are performed at 0°C. The tyrosinase activity of the supernatant is assayed by spectrophotometric measurement of formation of dopachrome following addition of excess substrate (dihydroxy phenylalanine in phosphate buffer pH 7.0) to the extract. Enzyme activity is expressed in tyrosinase units/gm. protein, one tyrosine unit being arbitrarily defined as that amount of enzyme equivalent to an increase in optical density at 480 mu of 1.0 per minute.

The tyrosinase activities of young adults of various genotypes grown at 25°C are as follows: wild type, 22; sable, 80; suppressor of sable, 204; suppressor of sable, sable, 210. In extracts of flies raised at 15°C there is proportionally greater enzyme activity. Comparisons on a time scale show differences in the enzyme acti-

vation process in various stages of development as well as an inverse correlation between tyrosinase activity and age. For example, the activity of sable stocks grown at 25°C is as follows: larvae, 316; pupae, 265; newly emerged adults, 80; aged adults, 22.

The tyrosinase activity of s/+ is intermediate between that of s/s and +/+. The kinetics of activation of extracts of s/+ is similar to that of mixtures of extracts of s/s and +/+. In both the *in vitro* and *in vivo* mixing, the activator behaves like that of the sable genotype whereas peak activity time is that of the wild type.

Kinetic studies of activation and inhibition, heat inactivation and pH experiments indicate that the various loci control the tyrosinase systems by exerting quantitative rather than qualitative effects.

LEWIS, JESSICA H., and C. C. LI. Genetic Considerations in Hemophilia A and B. *University of Pittsburgh, Pittsburgh, Pennsylvania, U.S.A.*

Two major types of hemophilia—A, due to a deficiency of the plasma protein "antihemophilic factor" (AHF), and B, due to a deficiency of a different protein "plasma thromboplastin component" (PTC)—are controlled by sex-linked, biochemically partially recessive genes. Type A is the classical hemophilia and comprises about 84% of the group. Extensive pedigrees—93 of Type A and 23 of Type B—were studied. In all 43% of A- and 52% of B-pedigrees showed the disorder in multiple sibships or generations.

Direct studies of sex ratio and hemophilic segregation were made by counting hemophilics' sisters' children and hemophilics' daughters' children. Among A-pedigrees there were 129 sisters' children sibships with a total of 452 individuals, of whom 233 (51.5%) were males and of these 77 were hemophilics (33% among males). Hemophilics' daughters' children (51 sibships) numbered 161. Of the 159 on whom the sex was known, 79 (49.7%) were males and, of these, 41 were hemophilics (51% among males). Among B-pedigrees there were 30 sisters' children sibships, containing 39 females, 37 males, and 6 sex unknown; 12 were hemophilics (calculated as 30% of male children). Hemophilics' daughters' children (22 sibships) numbered 74, of whom 49 (66%) were male. Of these, 22 were hemophilic.

Half of the sisters of hemophilics will be carriers. Thus, 25% of their male children would be expected to be hemophilics. Actually 33% hemophilics in A and 30% in B were found. Half of hemophilics' daughters' sons are expected to be hemophilic. In A 51% and in B 45% were. In the latter group the sex ratio showed an unexpected 66% males. If either the segregation for males or that for hemophilics among the males were more than 50%, as possibly suggested by the present studies, it would constitute another source for the supply of the hemophilia gene to compensate for its loss through selection in the population.

LEWIS, MARION, and BRUCE CHOWN. The Blood Groups of the Copper Eskimos. *Rh Laboratory, University of Manitoba, Winnipeg, Man., Canada.*

The blood of 348 Eskimos living in the Coppermine area was tested with the following antibodies, anti-A, A_1, B; M, N, S, s, He, Mi^a, Vw; P, Tj^a, C, C^w, c, D, E, e, V; K, k, Kp^a, Kp^b; Fy^a, Fy^b; Lu^a, Lu^b; Le^a; Di^a; Be^a, By^a; Yt^a and Zu, and

the saliva of most of them tested for the presence of A, B or H polysaccharide. The following antigens were not found: He, Mi^a, Vw; C^w, V; K, Kp^a; Le^a; Lu^a; Di^a; Be^a; By^a. All bloods tested reacted with anti-D; k, Kp^b; Lu^b; Yt^a; Zu. All persons tested were secretors of A, B or H polysaccharide. In the ABO system there was a high frequency of A_1; in the MNSs system only two M—N+ bloods were encountered; S was usually partnered with M; in the Rh system only R_1, R_2 and r appeared to be present, r being traceable to white ancestors; in the Duffy system there was a higher frequency of Fy^b than in any other native population we have tested. In family studies there were no discrepancies from expectations.

LEWONTIN, R. C. The Destruction of a Heterotic System by Natural Selection. *Genetics Faculty, North Carolina State College, Raleigh, N.C., U.S.A.*

It is probable that the maintenance of balanced polymorphism due to heterosis is contingent upon fluctuation of the environment causing alterations in the fitnesses of homozygotes and heterozygotes. In a fairly constant enviroment it would be possible to find genes more fit in homozygous condition than in heterozygous state, and such genes would be fixed by natural selection with a concomitant loss of polymorphism. This hypothesis was confirmed by the following set of experiments. A population of *Drosophila pseudoobscura* containing two third chromosome inversions, Arrowhead and Pikes Peak, was established under constant conditions of food and temperature. The frequencies of the inversions reached an equilibrium state at about 83% Arrowhead and remained in that balanced condition until about the twentieth generation of the experiment. Thereafter a sudden increase in the frequency of the Arrowhead inversion occurred, followed by a steady climb until the end of the experiment. In the twenty-sixth generation chromosomes were sampled from the population and four new populations were made up. These new populations contained either reselected Arrowhead and Pikes Peak chromosomes, original Arrowhead and Pikes Peak chromosomes, or combinations of reselected and original chromosomes. The two cages started with reselected Arrowhead strands showed a much more rapid rise in the frequency of Arrowhead than the two cages containing original Arrowhead strands. The latter two populations showed the same changes as did the original experimental population. The conclusion is that genetic changes occurred within the Arrowhead gene pool causing Arrowhead to be more fit in homozygous condition. It is this improvement that was responsible for the loss of the balanced polymorphism.

LI, C. C. *See* LEWIS, JESSICA H.

LI, H. W. Cytological Studies of Sugarcane and Its Relatives XV: Basic Chromosome Number of *Saccharum officinarum* L. *Institute of Botany, Academia Sinica, Taiwan, China.*

The euploid number of *Saccharum officinarum* L. is 80. By crossing noblecane variety Vellai ($2n = 80$) to *Sclerostachya fusca* A. Camus ($2n = 30$), and by

repeated back-crossing to the recurrent male parent, the chromosomes of sugarcane will be halved in each successive back-cross generation. Finally, in the third back-cross generation, only 10 sugarcane chromosomes are left in the hybrid along with 30 *Sclerostachya-Narenga* chromosomes. (The third back-crossing was done in Taiwan. *Narenga porphyrocoma* (Hance) Bor ($2n = 30$) was used instead of *Sclerostachya*.)

SG310/6 (B.C. 1) was found to have 72 chromosomes, 42 of which belong to sugarcane, the other 30 to *Sclerostachya*. This variety was obtained by back-crossing F_1 hybrid to *Sclerostachya*. Many PMC examined had 36 bivalents showing complete autosyndesis. The larger closed-type bivalents of *Sclerostachya* can be easily distinguished from the smaller open-type ones of sugarcane. One or two tetravalents were formed by the sugarcane chromosomes.

In the second back-cross generation clone SG363/4 was found to have 50 chromosomes. In the majority of the cases, 25 bivalents were found. Again the clone showed complete autosyndesis. One sugarcane tetravalent was sometimes found.

In the third back-cross generation, *Narenga* was used. Out of 15 plants 14 were found to have 40 chromosomes. (The one exception had 63.) There was almost perfect pairing between the chromosomes of *Sclerostachya* and *Narenga*, forming 15 closed-type bivalents. The 10 sugarcane chromosomes remained either as univalents or as 1–3 open-type bivalents. As many as 4 trivalents were found. It showed that 4 sugarcane chromosomes were partially homologous with 40 *Sclerostachya-Narenga* chromosomes. Sometimes, two tetravalents were found. These were composed of two sugarcane and two *Sclerostachya-Narenga* chromosomes.

From these data, it is conjectured that the ten sugarcane chromosomes are probably made up of two sets of five. Three chromosomes of each set are partially homologous to one another. At least four chromosomes of one set of five of one species are partially homologous to four of these from either *Sclerostachya* or *Narenga*. Initial hybridization between two unidentified species with $n = 5$, each leading to segmental allopolyploidy, and followed by two successive autopolyploidies, has probably resulted in the origin of *Saccharum officinarum* with 80 chromosomes.

Lieb, Margaret. Relationships between Mutation, Phenotypic Lag and Protein Synthesis. *Brandeis University, Waltham, Mass., U.S.A.*

The yield of induced bacterial mutants is markedly influenced by culture conditions before and after UV irradiation. Experiments on a tryptophane-requiring (try—) strain of *Escherichia coli* B/r have shown that in exponentially growing broth cultures, protein synthesis is required for the expression of induced try+ mutants. Incubation of irradiated try— cells in broth containing chloramphenicol (CML) allowed DNA synthesis, a small amount of protein synthesis, and the expression of only a small fraction of the try+ mutants that appear in the absence of CML. When the CML-grown cultures were subsequently placed in broth without CML, the majority of the expected mutants appeared. Our findings do not confirm those of Witkin (Cold Spring Harbor Symposium XXI, 1956) who found that growth in CML permanently prevented the expression of induced try+ mutants. We believe that Witkin's results may be attributed to the fact that exposure to CML increases the time required by try+ mutants to initiate growth. When mutants are assayed on plates that also support the growth of try— cells, there is competition between

mutants and non-mutants for protein precursors required by try+ for phenotypic expression.

In stationary phase cultures of try—, a large fraction of UV-induced mutations are expressed under conditions allowing no appreciable protein synthesis. This was not observed in exponential phase cultures grown in the same broth medium. The storage of intracellular precursors and their use in the synthesis of protein required for phenotypic expression may account for the observed "0-point" prototrophic mutants.

LIMA-DE-FARIA, A., PATRICIA SARVELLA, and ROSALIND MORRIS. Structural Differentiation of the Chromosome in Different Tissues. *Institute of Genetics, University of Lund, Lund, Sweden.*

Ornithogalum virens is a species with only three bivalents at pachytene. Chromosome I is the longest, chromosome II has a large knob, and III is the nucleolar chromosome.

In Diptera definite segments of the chromosome are known to show different structural features in different tissues. *O. virens* permits study of the same phenomenon in plants. Besides pachytene, the chromosomes can be identified at the prophase of the second microspore mitosis where they exhibit an equally distinct chromomere structure. Hence, the chromomeres of the same chromosome may be compared in different tissues. At pachytene, chromosome I has an average length of 109.0 microns and an average chromomere number of 105.3. At prophase of the second microspore mitosis this chromosome is 22.3 microns in length and, surprisingly, has only 24.3 chromomeres. The same relationship is true for the other chromosomes. The length decreases by about one-fifth at the microspore mitosis and the chromomere number decreases also by approximately one-fifth. The average distance separating the chromomeres at these two stages is approximately the same: 1.07 and 0.89 microns for pachytene and prophase of the second microspore mitosis respectively (analysis of the whole complement in 20 cells).

The knob of chromosome II which is very large at pachytene is, contrary to expectation on the basis of simple contraction of the chromosomes, very small at the microspore prophase and sometimes is not distinguishable. Two weakly stained segments in chromosomes I and II are also not easily recognizable at the microspore prophase. On the other hand chromosomes I and III show two large deeply stained segements at diakinesis which are not present either at pachytene or at the microspore prophase.

Definite segments of the same chromosome show different structural features in different tissues.

LINDEGREN, CARL C. Recombination in Bacteria. *Biological Research Laboratory, Southern Illinois University, Carbondale, Ill., U.S.A.*

Demonstration of Mendelian heredity requires that all the segregants of a hybrid fall into two equal contrasting classes representing the corresponding characteristics of the progenitors. Although the bacterial geneticist cannot recover all the progeny

under his experimental conditions, he has assumed a 1:1 gametic ratio for every segregating character. The assumption that the phenotype represents the genotype involves the corollaries that (1) every change in phenotype is due to gene-change; (2) cytoplasmic inheritance does not occur in bacteria, and (3) recombination of phenotype is equivalent to recombination of genotype. To the bacterial geneticist "recombination" means "recombination of genes"; to the classical geneticist it means "recombination of phenotypes," from which it may or may not be correct to infer the recombination of genes. If the genotype can be identified by the phenotype, the phenomenon of Dauermodifikation first described by Jollos (1921) must be disregarded. An example of Dauermodifikation is supplied by the white variant arising from the pink yeast. A direct experiment in Saccharomyces on the stability of the heterozygous diploid carrying the genes for adenine-independence and adenine-dependence reveals that genes may be much more unstable in the heterozygous than in the homozygous condition. This phenomenon produces results resembling the recovery of prototrophs. Bacterial geneticists have inferred that the failure of their data to conform to the pattern of chromosomal behavior found universally in other organisms means that a new and different kind of chromosomal mechanism is present in bacteria. The failure of the data from bacterial genetics to fit the standard meiotic apparatus may not be due to a different kind of meiotic apparatus or even due to a different kind of hereditary behavior but may be connected with difficulties routinely encountered in all genetic analysis.

LINDSLEY, D. L., C. W. EDINGTON, and E. S. VON HALLE. Sex-Linked Recessive Lethal Changes in *Drosophila melanogaster* Suppressed by the Y Chromosome. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

Thirty-two sex-linked recessive changes that are lethal or semilethal as XO males but surviving as XY males were produced and studied in *Drosophila melanogaster*. They are divisible into three groups on the basis of their *bobbed* constitution and the fertility of X/Y males.

Six are deficient for factors in the proximal heterochromatin, including *bobbed*, and are fertile as X/Y males. None of these is associated with a translocation, and crossing-over in the X appears normal. These X's disjoin normally from their homolog during oogenesis, but five of them exhibit abnormal disjunction from the Y chromosome during spermatogenesis characteristic of X chromosomes deficient for proximal heterochromatin.

Ten of the altered X's are fertile as X/Y males and are not *bobbed* deficient. Six of the ten exhibit drastically reduced crossing-over, but only two are associated with translocations. The lethal changes appear to localize randomly on the X. These and additional observations that several exhibit variegated phenotypes and two survive as homozygous females led to the hypothesis that these are position-effect lethals.

The remaining sixteen altered X's, which may also be position-effect lethals, are sterile as X/Y males and are not *bobbed* deficient. Twelve are associated with X-autosome translocations. Their effect on crossing-over ranges from a quite severe reduction to nearly normal. These lethals tend to be located proximally on the X but are not limited to that region. Disjunction of these X's from their homolog during oogenesis is highly abnormal; when the second X is Muller-5, the incidence

of primary non-disjunction is about 0.33. The curious correlation among male sterility, translocation, and abnormal disjunction is unexplained. The meiotic divisions of these males are normal; they seem to owe their sterility to abnormalities in spermiogenesis. Specifically, sperm head elongation is incomplete, but the degree is different for different stocks.

LINK, K. P. *See* BEDNEKOFF, A. G., *et al.*

LOPER, J. C. *See* HARTMAN, P. E., *et al.*

LOWRY, DOROTHY C., and I. MICHAEL LERNER. Stabilizing Selection for Shank Length Consequent on Directional Selection for Egg Production. *Poultry Husbandry Department, University of California, Berkeley, Calif., U.S.A.*

A line selected for long shanks was derived twenty years ago from a flock of Leghorns bred for egg production and viability (Heredity *5*:75). Subsequent behavior of shank length in the egg production line is now being investigated with special reference to reproductive fitness.

It has been established that over an interval of several generations after the truncation of the foundation stock, its mean shank length regained a stable level although no conscious selection was practised for this character. Its variance remained unchanged from that in the original population. During this period artificial selection appears to have favored birds with shank length greater than the mean, excepting those exceeding the mean by three or more standard deviations. Thereafter, once the mean had become stable, artificial selection favored birds with intermediate shank lengths. The variance of birds selected for reproduction throughout the whole period of study was consistently smaller than that of their whole generation.

Differences in reproductive fitness of birds of varying shank lengths can be accounted for entirely on the basis of selection for egg production and viability. That is to say there was no natural selection for either intermediates or extreme shank lengths among dams chosen to reproduce the production-bred flock. This was not the case in the line selected for shank length, in which natural selection began discriminating against extremes after some generations of selection, an effect which disappeared after suspension of selection.

LYON, MARY F. Some Evidence concerning the "Mutational Load" in Inbred Strains of Mice. *Radiobiological Research Unit, Harwell, Didcot, Berkshire, England.*

In studies of the effect of external mutagenic agents on animals knowledge of spontaneous mutation is useful. It has long been known that not all the ova shed

by a female mouse are represented by living young at birth, and it has usually been supposed that the embryonic elimination implied by this was largely due to an unfavourable uterine environment. However, Haldane in 1936 calculated, on the basis of certain assumptions, that the frequency of heterozygotes for recessive lethal genes in an inbred strain of mice should be approximately one.

If these assumptions were correct a large proportion of the embryonic death in inbred lines of mice would be due to the segregation of recessive lethals. To test this hypothesis reciprocal crosses were made within and between three inbred strains: CBA/H, C3H/H and 101/H. Pregnant females were dissected and the proportion of embryos which had died between the time of implantation and 13½–15½ days' gestation was noted.

In crosses within strains the rate of embryonic death was consistently higher than in crosses between strains, showing that a proportion of the deaths were "recessive" in type. It is suggested that these deaths are due to the segregation of recessive lethal genes which had arisen by mutation within the strain, and the results are compared with those expected on the basis of Haldane's calculations.

MacDonald, M. A. Seasonal Growth Relationships in Aberdeen Angus × Jersey Crossbred Cattle. *Experimental Farms Service, Canada Department of Agriculture, Lethbridge, Alberta, Canada.*

During the late winter of 1953 twenty-one calves sired by purebred Aberdeen Angus bulls and out of commercial Jersey cows were brought to Ruakura Animal Research Station. Bucket feeding commenced when calves were 2 days of age and each was fed according to Ruakura recommendations. At 8 weeks of age 7 bull calves and 4 heifers were weaned and the remaining 6 males and 4 heifers at 18 weeks. Males were castrated at ages ranging from 14 to 17 weeks. Growth rate was measured by live weight gain and anatomical measurements until the animals reached 30 months of age. Data were analysed by Doolittle's method of least squares analysis, Brody's method for determining instantaneous growth constants and analysis of variance. The following results were obtained. (1) Steers gained 133 pounds more than heifers during the 30 month experimental period. This difference was highly significant ($P < .01$). (2) Age at weaning did not affect weight gain during either the first year or the entire experimental period. (3) Growth rate, measured by instantaneous growth constants for growth periods between ages of 1 and 52 weeks, decreased steadily with age for all groups. (4) Growth rates of all groups of crossbreds equalled that of purebred Aberdeen Angus cattle at Ruakura and exceeded that of range beef steers in Texas and feedlot steers fed a moderate T.D.N. ration in Missouri. (5) Anatomical measurements indicated that the period of pasture shortage during the second year did not suppress skeletal growth but did suppress liveweight gain and degree of fill. (6) Variation in liveweight within seasons and within groups was sufficiently large to prevent expression of statistically significant growth relationships between seasons. Relative seasonal gains could not be predicted.

On the basis of this and other studies, crossbred calves for veal and liveweight beef production are recommended. If a pasture shortage necessitates a reduction

in numbers carried beyond the veal stage, steers should be selected preferentially for retention.

MAGNI, G. E. Effect of Natural Selection on a Cytoplasmic Character in *Drosophila bifasciata. Istituto di Genetica, Università, Pavia, Italy.*

In *Drosophila bifasciata* the character "sex-ratio" is transmitted maternally and is due to extra chromosomal determinants. The frequency of "sex-ratio" females found in nature suggested the hypothesis that the permanence of the cytoplasmic character in natural populations can be assured by a selective advantage of the s.r. individuals over the normal ones. The behaviour of such character has been, therefore, studied under severe natural selection pressure in laboratory populations in which s.r. females were in competition with normal flies.

S.r. individuals under our experimental conditions show a definite selective advantage which causes a progressive decrease in the proportion of normal flies until these are present in approximately 10% of the population. After several generations, however, the trend is reversed and the relative number of normal females increases considerably. An analysis of these populations showed that the variations in the frequency of the s.r. females are due to evolutionary changes occurring both in the genotype and the cytoplasm and resulting in different equilibrium values at different times.

MAINX, F. Population Genetics of *Drosophila* Species on the Liparic Islands. *Institut für Allgemeine Biologie der Universität Wien, Vienna, Austria.*

In 1956–7 research was carried out on the influence of insular isolation on the genetic structure of *Drosophila* populations on the Liparic Islands in the Thyrrenean Sea. The distribution of some species of the genus is in accordance with the warm climate of the Mediterranean, but compared with results on the continent shows disparities caused by isolation.

One main subject was concerned with the frequency and the allelism of recessive lethals in II-chromosome of *D. melanogaster*, studied by crossing wild males with a stock Pm/LCy. The frequency of lethals is approximately the same in spring and in autumn, about 25%. This figure is low compared with continental populations, for instance, Palestine with 32%, Florida with 60% lethals in II-chromosome. The degree of allelism was about 0.72% in autumn and in spring as much as about 2.38%. These figures are high compared with continental statements, for instance 0.33% in Florida, 0.34% in autumn and 0.83% in spring in Palestine. Although these figures are not yet final, the differences are significant.

Another subject was the chromosomal polymorphism of *D. subobscura.* Qualitatively there are some differences from the populations in Central Europe. The degree of structural heterozygosity seems to be no smaller than in continental populations. The effect of heterosis is so powerful that it keeps up a high degree of heterozygosity against the genetic drift.

MAJUMDER, S. *See* BREWBAKER, J. L.

MAKINO, SAJIRO. Alteration of the Stemline Chromosomes in Transplantable Ascites Tumors of Rats. *Zoological Institute, Hokkaido University, Sapporo, Hokkaido, Japan.*

The chromosome cytology of transplantable ascites tumors in rats and mice have afforded available data which support the reality of the stem-cell hypothesis that each neoplastic population contains a characteristic stem-line (or lines) of tumor cells which function as the primary contributors to the neoplastic growth maintaining the genetic pattern of each tumor. The tumor cells most frequently occurring with characteristic chromosome number-mode together with a specific ideogram form a stem lineage (or lineages). Each tumor is clearly distinguishable from others, as well as from the ordinary tissue, by its stemline ideogram (or ideograms).

The drastic applications, cold storage, heteroplastic transplantations and some other experimental procedures have generally failed to induce any change of either the stemline ideogram or its genotype. In the light of the recent investigations, however, it has been shown that the stability of the stem-line, while it is very pronounced, is not always permanent, and tends to undergo transition after its origin during serial transfers with the rise of a new type of tumor. The shifts may involve structural rearrangements and other adjustments of the stemline chromosomes. If such a type of cells newly arisen were superior in competitive ability to the population of original type of cells, the cell population as a whole would shift into the new cell type after several generations of selections, and such changes will produce a change of the genotype of the tumor with the shifts in its property. It is interesting that the rise of a new type of the tumor takes place in close association with alteration of the stemline chromosomes.

MALOGOLOWKIN, CHANA, and D. F. POULSON. Sex-Ratio in *D. willistoni* and *D. paulistorum. Faculdade Nacional de Filosofia, Universidade do Brazil, Rio de Janeiro, Brazil,* and *Department of Zoology, Yale University, New Haven, Conn., U.S.A.*

Abnormal "sex-ratio" in *Drosophila* was first reported by Magni (Inst. Lombardo Sci. Lett. *75*:3–23) in *D. bifasciata* and by Cavalcanti and Falcao (Caryologia *6*:1233–5) in *D. prosaltans.* It is transmitted from mothers to daughters in the form of progenies consisting mainly, or exclusively, of females. When crossed to males from a variety of strains giving normal proportions of the sexes, "sex-ratio" females continue to produce mainly daughters. Similar cases of "sex-ratio" have been described by us in *D. willistoni* and in *D. paulistorum* (Malogolowkin & Poulson, Science *126*: 32; Malogolowkin, Genetics, in press). These two species are widely distributed geographically, being abundant in South and Central America (Dobzhansky, Burla & Cunha, Amer. Nat. *84*: 229–46; Dobzhansky, Evolution *2*: 280–93). Although large numbers of individuals have been collected over a period of years these are the first instances of "sex-ratio" in these sibling species.

Single females each of *D. willistoni* from Jamaica and of *D. paulistorum* from Colombia gave rise to "sex-ratio" strains which were shown to carry a cytoplasmic factor responsible for the condition (Malogolowkin, 1958). The original progenitors of these "sex-ratio" strains evidently carried genes favorable to the multiplication of the cytoplasmic agent which leads to the death of all XY zygotes bearing it and so brings about the unisexual progenies. This is also true of certain laboratory

of strains from certain different localities the "sex-ratio" effect was reduced, and after a few backcrosses to such males, the normal proportions of sexes appeared. In such cases the cytoplasmic agent is irreversibly lost, just as *kappa* in *Paramecium aurelia* (Sonneborn, 1943) disappears in the unfavorable genotype.

To determine whether as in the case of the CO_2 sensitivity factor in *D. melanogaster,* (L'Heritier & De Scœux, Bull. Biol. France Belg. *80*: 171–227) the "sex-ratio" agent of *D. willistoni* might be transferred by injection into other strains we carried out a series of injections of ooplasm from the abnormal male zygotes into the abdomens of young virgin females of normal strains (Malogolowkin & Poulson, Science *126*: 32; Malogolowkin, Poulson & Wright, Genetics, in press). Transfer was accomplished and "sex-ratio" has been established even in a strain genotypically resistant to the cytoplasmic agent. Injection of "sex-ratio" ooplasm into adult males is always lethal within a few days. Available evidence suggests that the "sex-ratio" agent is limited to the female germinal line.

MANGELSDORF, PAUL C. A Genetic Reconstruction of the Ancestor of Maize. *Botanical Museum, Harvard University, Cambridge, Mass., U.S.A.*

Recent studies of fossil maize pollen from the Valley of Mexico and of prehistoric maize cobs from once-inhabited caves in Mexico, New Mexico, and Arizona indicate that the ancestor of cultivated maize was a form of pod corn with small ears, bearing tiny seeds enclosed in glumes. In an attempt to reconstruct this ancestor by combining primitive characteristics still existing in cultivated maize, pod corn, which has covered seeds, was crossed with pop corn which has small ears and seeds. The maize produced by this hybridization not only has several of the principal characteristics found in the prehistoric maize, but it also exhibits other traits which are probably ancestral. The genetically reconstructed ancestral form is a many-stalked plant bearing its ears in the higher, more slender regions of the stalk which can support only small ears. Ears borne at these high positions have few husks and these often flare open at maturity allowing the exposed ear to disseminate its small chaff-covered seeds. Both ears and tassels bear both male and female flowers and in this respect resemble the inflorescences of *Tripsacum*, a wild relative of maize.

In the course of evolution under domestication an ancestral form of this type has changed in several ways to produce the cultivated maize plant of today: (1) the many-stalked plant has become single stalked; (2) the ear has changed its position to a lower and stronger part of the stalk which is capable of bearing larger and heavier ears; (3) in this lower position the ears bear only female flowers and produce only grain; (4) in this lower position the ear has more numerous husks and is completely enclosed by them; (5) the covering of the individual grains has been lost.

Most of these evolutionary changes, which render modern maize unique among the cereals in its botanical characteristics, are in part the product of a single mutation at the *Tu* locus on chromosome 4.

MANN, T. J. *See* MATZINGER, D. F.

MANNA, G. K., and S. G. SMITH. Adaptive Chromosomal Polymorphism and Inter-Relationships among Bark-Weevils of the Genus *Pissodes* Germar. *Forest Insect Laboratory, Department of Agriculture, Sault Ste. Marie, Ont., Canada.*

Examination of males and females of 11 North American species of *Pissodes* has established the following diploid chromosome numbers: *strobi, engelmanni,* and *sitchensis* = 34; *approximatus* and *canadensis* = 30 — 34; *affinis, dubius, fasciatus,* and *radiatae* = 30; *yosemite* = 28; and *terminalis* = 28 (♀) and 29 and 32 (♂). The variation in chromosome number is chiefly due to changes ("fused" *versus* "unfused") in the 3 largest pairs of chromosomes, which are labelled A, B, and C. Thus in *yosemite* the first spermatocyte metaphase contains 3 large bivalents usually as rings, the 30-chromosome group has 2 rings, while there are no rings in the 34-chromosome group other than the asymmetrical one formed by the X and Y in all species.

Extreme intraspecific chromosomal polymorphism has been encountered in *approximatus* and *canadensis*. About 570 adults, mostly males, of *approximatus* from different localities in Ontario have shown the 9 karyotypes expected as a result of heterozygosity for the A and B fusions. The population frequencies suggest that (single and double ??) fusion homozygotes are better adapted than other polymorphs. In *canadensis* it appears, from 62 adults studied, that in some populations of Manitoba the reverse is true. Further, in both species, a medium-sized heteromorphic autosomal bivalent is occasionally found.

Presumably *terminalis* can be heterozygous for A, B, and C fusions, but surprisingly, instead of the expected 27 polymorphs, 49 of the 50 males examined were heterozygous for A and homozygous for fusions B and C, the fiftieth ($2n$ = 32) being heterozygous for both A and B and homozygous for unfused C.

A detailed analysis of the karyotypes and their frequencies in natural populations has been made and the evolutionary significance is discussed.

MANUNTA, CARMINA. Biochemistry and Genetics in Silkworms (*Bombyx mori*): Chromatographic Patterns and Nitrogen Metabolism. *Istituto di Biologia e zoologia generale, Università Sassari, Sassari, Italy.*

Substances which are fluorescent in ultraviolet light are present in the cocoons of silkworms and in their eggs, larvae and pupae. Some of these substances are derived from the food—mulberry leaves for *Bombyx mori*, and ailanthus for *Philosamia cynthia*; some others appear to be endogenous.

Paper chromatography, applied to the pupae of *Bombyx* of several races, shows sharp racial differences in the series of spots: 4–9 in ammonium propanol, and 6–12 in acetic butanol.

Notwithstanding great individual variability inside each strain, we can recognize three groups of strains exhibiting complete, incomplete, or intermediate fluorescent patterns. In many strains a differential pattern has been observed in the two sexes; and comparison between patterns in extracts of pupal body and caecum (excretory ampulla) and several layers of the silk cocoon shows a differential distribution of the fluorescent material. The injection of silkworms of many breeds with four synthetic pterins has shown, in a few strains, a differential migration from blood to silk for each fluorescent substance introduced, single or mixed: especially for xanthopterin and leucopterin.

The application of chromatographic methods—on cellulose column and on paper—to a mass of cocoons of several breeds of *Bombyx* has effected the separation of many fluorescent substances—no less than thirty—from the silk. For eight of these it has been possible to determine the absorption curve in ultraviolet light. It is very characteristic—unlike those obtained for natural or synthetized pterins—and points to four different chemical groups, at least. While chemical identification is considered an important task for future researches, investigations are under way on the specific differential permeabilities and their Mendelian behaviour as a connecting link between previous researches on physiogenetics of pigments and nitrogen metabolism.

MARCOU, DENISE. Sur le déterminisme de la sénescence observée chez l'Ascomycète *Podospora anserina*. *Faculté des Sciences de Paris, Laboratoire de Génétique, Paris, France.*

Les souches de l'ascomycète *Podospora anserina* cultivées dans des conditions où leur croissance est continue n'ont pas une longévité infinie. Après un temps où la croissance est constante, celle-ci diminue rapidement, l'allure du mycelium devient anormal, la croissance s'arrête et les extrémités des filaments meurent. On dit que la souche est sénescente.

Toutes les souches meurent ainsi, mais elles peuvent avoir des longévités très différentes (de quelques jours à plusieurs années). La longévité de chaque souche peut être représentée par une courbe, caractéristique de chaque souche dans des conditions données, mais dont l'allure générale est toujours la même: un palier plus ou moins long suivi d'une droite descendante à pente variable.

La longévité d'une souche dépend de nombreux facteurs: température, éclairage, richesse du milieu de culture, génotype.

L'origine cytoplasmique de la sénescence a été mise en évidence par la technique des croisements réciproques. La sénescence peut en effet être transmise aux descendants, par l'élément femelle et par lui uniquement. Par ailleurs, on n'observe de ségrégation du caractère ni dans l'asque, ni même dans le périthèce.

Des expériences de micromanipulation ont confirmé ce résultat. La sénescence se révèle infectante par anastomose bien que sa propagation dans un hyphe soit assez lente. Or on sait que chez cet organisme les anastomoses permettent un échange uniquement cytoplasmique entre les hyphes.

Une hypothèse particulaire peut rendre compte de ces résultats. Il peut s'agir soit de la multiplication anormalement rapide d'un élément normal de la cellule, soit de la multiplication d'un élément n'existant pas sous cette forme dans les souches normales.

MARCOVICH, HERBERT. Recherches sur la mutabilité spontanée d'*Escherichia coli* B. *Laboratoire Pasteur de l'Institut du Radium, Paris, France.*

L'étude du mécanisme de la mutabilité spontanée chez les bactéries est génée par la présence de nombreux mutants dans le milieu de culture. En utilisant la technique de Davis à la pénicilline il est possible dans une population de bactéries auxotrophes de faire décroître considérablement le taux des prototrophes. Ainsi, sur

la souche d'*Escherichia coli* B h^- qui a besoin d'histidine comme facteur de croissance le taux des h^+ qui varie normalement entre 10^{-7} et 5.10^{-7}, peut être amené à une valeur inférieure à 10^{-9}. Il est allors possible de déceler avec précision l'apparition de prototrophes nouveaux.

Cette technique a été appliquée à l'étude de la mutabilité de bactéries dans un état de non-prolifération.

Les princiaux résultats suivants ont été acquis. (1) Aucune mutation nouvelle n'apparait si les bactéries sont gardées à l'état congelé à −25°C ou à l'état liquide vers +2°C. (2) Aucune mutation nouvelle n'apparait si les bactéries sont incubées à 37°C dans un milieu minimum dépourvu de source d'énergie (glucose). (3) Des mutations apparaissent à 37°C en présence de glucose à un taux de l'ordre de 10^{-9} prototrophes par bactérie et par heure. La mortalité dans nos conditions expérimentales est nulle.

Ces résultats sont discutés.

MARTIN, G. A., and A. E. BELL. Response to Selection for Adult Weight and Observed Changes in Reproductive Fitness in *D. melanogaster*. *Poultry Science Department, Purdue University, Lafayette, Indiana, U.S.A.*

From each of 2 independent samples drawn from a synthetic population (synthesized from 12 inbred lines) of *D. melanogaster*, a large and a small adult body weight line was selected for 15 generations. As Falconer observed in mice (J. Genet. *51*: 470–501), response to selection was asymmetrical toward small body weight in the early generations. The early asymmetry was partially reversed by rapid progress in the large lines during the mid-portion of the experiment. All lines showed decreased response after generation eleven. The coefficients of regression of body size on generation of selection for generations 1–6, 5–11, and 10–15, respectively, were +1.78, +17.69, +7.19 and +16.24, +19.35, +17.35 μg. for the two large lines and −29.92, −13.62, −11.20 and −10.93, −11.90, −7.48 μg. for the two small lines.

Daily egg production, length of eggs in micrometer units of microscope eyepiece, and percentage of eggs producing adults were measured for each of the lines as functions of reproductive fitness. The coefficients of regression of egg production on generation of selection were −5.77, +.71, −1.19 and −3.35, −1.08, +.33 eggs (for generations 1–6, 5–11, 10–15 respectively) for the large lines and −8.37, +2.47, +2.61 and −2.54, −3.45, −2.02 eggs for the two small lines. The coefficients of regression of egg length on generation were −.13, −.05, −.09 and −.08, +.03, −.05 units for the two large lines and −.41, −.07, −.24 and −.05, −.22, −.14 units for the two small lines. Coefficients of regression of percentage of eggs producing adults were −2.01, −1.34, +1.27 and −5.37, −2.79, −3.11% for the two large lines and −4.88, −.07, −1.80 and −.81, −4.48, −.65% for the two small lines.

The fact that all lines have negative regressions for the three fitness traits during generations 1–6 while body size was moving in opposite directions, indicates that neither pleiotropy nor tight linkage of genes influencing body weight and the other traits have paramount effects in these populations. The role of inbreeding will be presented. (This work was supported by grants from the Rockefeller Foundation and the National Science Foundation.)

MATSUI, TETSUO. *See* SMITH, JIMMIE B.

MATSUMOTO, KENZO. Morphology of *Aegilops* F_1 Hybrids with Reference to Genome Differentiation. *Biological Laboratory, Department of Natural Sciences, Osaka University of Liberal Arts and Education, Tennoji, Osaka, Japan.*

In relation to the genome constitutions, various ear characters have been studied morphologically in *Aegilops* F_1 hybrids of about 90 different combinations. The ear characters were color, shape, type of articulation, rudimentary keel of empty glume, hairiness on glumes, and number of awns or teeth of lateral and apical spikelets.

In nine diploid species, or analysers after Kihara (1954), the respective characteristics correspond to the respective genomes, while in the tetraploids their specific characteristics are controlled by the second genomes involved in combinations with those of the analysers, and similarly for the third genomes of the hexaploids.

Based on the present studies it will be shown that the second genomes of the tetraploids are morphologically more differentiated than those of the analysers, but the third genomes of the hexaploids show little or no differentiation.

Details will be discussed in regard to each of the above-mentioned items of the ear characters.

MATSUMURA, S., M. MURAMATSU, and S. SAKAMOTO. Genome Analysis in *Agropyron*, a Genus Related to *Triticum*. *National Institute of Genetics, Misima, Japan.*

The genus *Agropyron* comprises three sections, *Euagropyron*, *Elytrigia* and *Roegneria*. Intrasectional hybrids are easily obtained, but intersectional crosses are difficult. Hybridization experiments with *Triticum* and *Agropyron* showed that the easiest crosses were those between the Emmer or Dinkel group and section *Elytrigia* and it was difficult to obtain a hybrid between the latter and the Einkorn group, while crossing of *Triticum* with *Roegneria* was unsuccessful. In the sterile F_1 between *A. glaucum* (= *A. intermedium*, EEFFJJ) and several Emmer species (AABB) varying chromosome conjugations $3_{II}+29_I \sim 12_{II}+11_I$ (mode, $(7\text{–}8)_{II}$) were observed. The F_1, *T. Timopheevi* (AAGG) $\times$ *A. glaucum*, was completely sterile and showed various conjugations $2_{II}+31_I \sim 8_{II}+19_I$ (mode, 5_{II}). In the sterile F_1 between *A. glaucum* and two Dinkel species (AABBDD) various kinds of conjugation $2_{II}+38_I \sim 13_{II}+16_I$ (mode, 6_{II} & 11_{II}) were found. Crossing of *A. glaucum* with *Aegilops longissima* (S^1S^1), *Ae. cylindrica* (CCDD) and *Ae. variabilis* ($C^uC^uS^vS^v$) was successful. All hybrids were completely sterile and showed various kinds of conjugation, namely, $1_{III}+5_{II}+15_I \sim 10_{II}+8_I$ (mode, 8_{II}) in the hybrid with *Ae. longissima*, and $5_{II}+25_I \sim 10_{II}+15_I$ (mode, 6_{II} & 8_{II}) in the hybrids with the tetraploid species. Judging from the occurrence of six to eleven bivalents in these hybrids, allosyndesis of several chromosomes between the genomes of *Triticum* or *Aegilops* and those of *Agropyron* may be assumed, which might for the most part belong to the *Triticum* B- or *Aegilops* S-genome, partially homologous with the J-genome of *A. glaucum*. Moreover, the possibility of autosyndesis of several chromosomes between the genomes of *A. glaucum* is indicated.

According to the hypothesis of McFadden and Sears (1946), *A. triticeum* should have the B-genome. It is well known that all chromosomes of the B-genome in *Triticum* have median or submedian constriction. But all seven chromosomes of *A. triticeum* show subterminal centromeres, while the diploid *A. elongatum* and *A. cristatum* have chromosomes with median or submedian constriction.

MATZINGER, D. F., T. J. MANN, and H. F. ROBINSON. Estimates of Genetic Variability in Flue-Cured Varieties of *Nicotiana tabacum*. *North Carolina State College, Raleigh, N.C., U.S.A.*

Little evidence has been accumulated on the type and magnitude of genetic variance in *Nicotiana tabacum*. Previous estimates of sizable amounts of additive genetic variance but little dominance variance were obtained under the assumption of no epistasis. A cross between C139, a high yielding variety, low in flavor and aroma, and Hicks, intermediate in yield and of good quality, was studied for the nature of the inheritance of certain agronomic and chemical characters. Eight F_2 plants were assigned at random to a set, four being designated as male parents and four as female parents. Each male was crossed to each female making a total of 16 crosses per set, and all parent plants were selfed to give 8 F_3 families. The progenies of crosses from 10 of these sets were evaluated with each set comprising a block in the field with replication within a block. From a comparison of variances of the intercrosses and the selfed progeny, and from the covariance of F_2 plants in crosses versus selfed progeny performance, estimates of additive genetic, dominance, and additive $\times$ additive epistatic variances were obtained under the assumption of no other types of epistasis. Characters for which data are available include leaf yield, value per acre, value per 100 pounds, date to first flower, plant height, number of leaves, number of suckers, length of largest leaf, width of largest leaf, and percentage of nicotine. Predicted genetic advance is considered from selecting the superior full-sib families and intercrossing to create the next generation of material.

MAYNARD SMITH, J. The Genetics of Longevity in *Drosophila subobscura*. *Department of Zoology, University College, London, England.*

Adult life span of *Drosophila subobscura* at 20°C has been measured for nine inbred lines, for two kinds of F_1 hybrids between inbred lines, for the F_1 from wild females caught in southern England, and for the F_1 and F_2 from wild females caught in Galilee.

There were large differences between inbred lines, but most inbred populations had a lower mean life span than outbred ones, and were more variable not only than the F_1 hybrids but also than the populations derived from wild females.

Significant sex differences in longevity were found in six of the nine inbred lines, in one of the two F_1 hybrids, and in the Galilee population. In all, four sex differences in each direction were found. This shows the importance of genes with sex-limited effects on longevity, i.e., with different effects on males and females.

In the Galilee population, correlations between relatives of the same sex were higher than between relatives of unlike sex, suggesting that genes with sex-limited effects were responsible for an appreciable part of the differences between families in the Galilee population.

Females which have no ovaries, or which are unmated and so lay fewer eggs, or which are kept at 30.5°C soon after emergence for a period of days sufficient to cause regression of the ovaries live longer than do normal mated females. Thus in females the rate of ageing depends on the rate of egg-laying. It follows that the causes of ageing are in part different in the two sexes, and it is therefore not surprising to find that genes with sex-limited effects on longevity are important.

MAZOTI, LUIS B. Inherited Preferential Somatic Segregation in *Zea* Conditioned by the Cytoplasm. *Instituto Fitotécnico de Santa Catalina, Buenos Aires, Argentina.*

The pure line carrying teosinte cytoplasm, genotype pl/pl (no anthocyanin) was backcrossed four times to the genotype PL/PL (anthocyanin bearing) carrying *Zea* cytoplasm: 86 individuals resulted, 37 of which were of the genotype Pl/pl and 49 PL/PL. Theoretically, we would have obtained 5 heterozygotes and 80 homozygous dominants (15:1 ratio). The heterozygote PL/pl backcrossed to the homozygous recessive should have given a ratio of 1 PL:1pl. The actual values were PL 1,022 and pl 1,296. This discrepancy suggests a preferential segregation. To eliminate the possibility of a point mutation the line in question was backcrossed using a genetic marker sm (salmon-coloured stigma) located 10 map units from pl. In the backcross PL/pl sm/sm × pl/pl sm/sm, we obtained 99 PL/sm: 8 PL/sm: 11 pl/sm and 125 pl/sm with an excess of pl associated with gene sm. These results would indicate that this is not a point mutation.

The homozygous dominant PL/PL in *Zea* cytoplasm was backcrossed using the same genetic marker as before. In contrast, no excess of pl from the expected 1:1 ratio was obtained indicating that the cytoplasm is responsible for preferential segregation. In addition, heterozygous plants PL/pl on teosinte cytoplasm showed mosaics with non-purple regions in the panicle. The pollen from these regions was used to pollinate plants (pl/pl genotype) with the following ratio obtained, 77 pl/pl: 54 PL/pl. When pollen from purple regions was used, the ratio obtained was 22 pl/pl: 27 PL/pl. This sporadic instability which has been seen previously in this homozygous line (introduced to the Argentine from Cornell University, U.S.A.), and the high frequency of small mosaics in the anthers which would show sterile pollen when the heterozygous genotype was introduced into teosinte cytoplasm, point to a more complex hypothesis which requires cytological studies for its elucidation.

MCDONALD, DANIEL J. A Study of Mutations affecting the Viability of *Tribolium confusum*. *Department of Biology, Dickinson College, Carlisle, Pennsylvania, U.S.A.*

Two new mutations of *Tribolium confusum* have been isolated which have an adverse effect on viability. The first of these, designated "split" (abbreviated *sp*), was obtained from an inbred line of *Tribolium confusum*. It is an autosomal recessive which, in the homozygous condition, shortens and separates the elytra, exposing the posterior third of the abdomen. The expression of the gene does not seem to be dependent upon the degree of inbreeding. The fertility of the mutants is lowered as indicated by a high percentage of infertile pair matings (about 37%),

and the viability is calculated to be about 88%. It is possible that the decreased fertility may simply be the result of inbreeding, but this has not been determined.

The second mutation, designated "Striped" (abbreviated *St*), is the first instance of a sex-linked lethal mutation to be reported in *Tribolium confusum*. This mutation, which appeared in a black strain of *confusum*, modifies the pigmentation of the elytra. In heterozygous females, a white stripe runs lengthwise along each elytron which is ordinarily solid black. In homozygous mutant females and males, the entire elytra is white. These males and females are completely nonviable and the females seem to be almost completely sterile. Heterozygous females, on the other hand, are viable and fertile. The viability of the white males and females is about 14%. Characterizing mutant beetles is beset by two difficulties. First, the stripes tend to fade and become almost indistinguishable after two weeks. Second, white individuals will occasionally have a small median strip of pigment and might be mistaken for striped beetles.

McGinnis, R. C., and A. B. Campbell. Fertile Triple Monosomics from a *Triticum vulgare* Cross. *Cereal Breeding Laboratory, University of Manitoba, Winnipeg, Man., Canada.*

A cross between a nullisomic and normal wheat plant usually produces only monosomic progeny. However, of eight plants from the cross, Redman nullisomic XXI $\times$ Prelude, only one plant was monosomic ($20^{II}1^{I}$), two were double monosomics ($19^{II}2^{I}$), while five were triple monosomics ($18^{II}3^{I}$). All the deficient plants were vigorous and partially fertile. Furthermore they were almost as tall and early as the normal Redman-Prelude hybrids having 21^{II}.

Ten F_2 plants from each of one selfed double and three selfed triple monosomics were grown and cytologically analysed. In general, the parental chromosome constitution predominated. Fertility counts were made on all F_2 plants. The monosomics averaged 62% fertility (ranging from 46–81%); the double monosomics averaged 38% (ranging from 1–73%); and the triple monosomics averaged 23% (ranging from 0–39%). One F_2 plant from a triple monosomic parent was doubly monosomic and singly nullisomic ($18^{II}2^{I}$), indicating that both male and female chromosome-deficient gametes functioned. This plant also appeared normal in growth and had 24% fertility, approximating the triple monosomic average.

An F_3 population from high- and low-fertility representatives of each deficient F_2 class was analysed. It was observed that the progeny tended to approach the full 21^{II} chromosome complement, although parental chromosome constitutions occurred with sufficient frequency to permit maintenance of the deficient stocks.

McLaren, Anne, and Donald Michie. An Effect of the Uterine Environment upon an Inherited Skeletal Character in the Mouse. *Royal Veterinary College, University of London, London, England.*

The number of lumbar vertebrae in the mouse is known to be under genic control. The same character shows a strong matroclinous tendency in reciprocal crosses between contrasting types. Using the technique of fertilised egg transfer, we have shown that the maternal effect is mediated through the uterine environ-

ment rather than through the cytoplasm of the egg. The only character previously known in any mammal to be affected by the uterine environment is body size. Number of lumbar vertebrae in mice is not related to body size, and the uterine influence cannot therefore be a simple nutritional one. Further work is planned to see whether the effect persists, as a kind of "somatic inheritance," for more than one generation.

MECHELKE, FRIEDRICH. The Timetable of Physiological Activity of Several Loci in the Salivary Gland Chromosomes of *Acricotopus lucidus*. *Institut für Kulturpflanzenforschung der Deutschen Akademie der Wissenschaften zu Berlin, Gatersleben, Germany.*

The salivary gland of the chironomid *Acricotopus lucidus* consists of 3 lobes: anterior, lateral and main. The secretion of each lobe is specific, as indicated by differences in the colour and consistency of the saliva. Every cell of the gland contains in its nucleus 3 giant chromosomes, which are easily distinguished by their patterns of bands. There are 12 distinct loci in the set of chromosomes, which are able to form puffs and balbiani-rings. The formation of such a functional structure indicates that the said locus is in full activity of genephysiological metabolism. If the structures of these 12 loci in the chromosomes of the various lobes during the development from the fourth larval instar through the prepupal to the young pupal stage are compared, it is obvious that each locus is not activated in each lobe and that the duration of activity is not simultaneous for all the loci. Owing to the specifically structural variations and changes of each locus, it is possible to present a detailed timetable of their physiological activity. In the anterior lobe especially the structural changes of the activated loci and the qualitative changes of the saliva produced demonstrate strikingly the parallel relations between the activity of loci and the specificity of metabolism.

The phenomenon of local and temporal limited formation of puffs and balbiani-rings may be considered as an enlargement of surface by unfolding the submicroscopic structure of the locus concerned. The unfolded surface represents the basis for catalytic reactions by which the genes deliver their specific products to the cell-metabolism. There are some parallel features between functional structures of giant chromosomes and interphasic structure of mitotic chromosomes. Characteristic for both is the enlargement of surface during the period of greatest metabolic activity.

MEHL, H. G. *See* EHLING, U.

MELACRINOS, A. *See* PRIADCENCU, AL., *et al.*

MELBER, D. *See* PRIADCENCU, AL., *et al.*

METTLER, LAWRENCE E., and MICHAEL A. BENDER. Chromosome Studies and Their Evolutionary Significance in New World Primates. *Department of Biology, The Johns Hopkins University, Baltimore, Maryland, U.S.A.*

It is generally agreed that the infraorder Platyrrhinae (New World monkeys) are composed of 2 families; Cebidae and Hapalidae. The Hapalidae may be divided into 2 sub-families; Hapalinae and Callimiconinae. Karyotypes have been obtained for 7 of the 9 genera of Cebidae, and preliminary studies made on the other 2. Karyotypes have also been obtained for a representative marmoset and a representative tamarin from the Hapalinae. Preliminary studies have been made on *Callimico*, the only genus in the Callimiconinae. Cytological observations were made on tissue-cultured somatic cells. Some observations were also made on bone marrow to verify the reliability of tissue-cultured material for cytological investigations. The highest diploid number found in Cebidae was 54 (genus *Cebus*), the lowest 34 (genus *Ateles*). In general, species with high chromosome numbers have a greater number of telocentric chromosomes; those with a low number have fewer telocentrics. Thus *Cebus* has 14 pairs of telocentrics, *Ateles* only one, excluding the sex chromosomes. The morphology of the X-chromosome varies as to size and position of the centromere in different species. The Y-chromosome is typically a very small telocentric. Species with low chromosome numbers (e.g., *Ateles*) are generally considered more specialized, those with high chromosome numbers (e.g., *Cebus*) less specialized on morphological and ecological criteria. It is proposed that a basic diploid chromosome number (in excess of 50) occurs in the Platyrrhinae. Chromosome evolution in the New World primates appears to have involved an alteration of the basic complement by such mechanisms as centric fusions and pericentric inversions, and has not involved polyploidy.

(This work was supported by a research grant from the National Science Foundation to Professor Wm. L. Straus, Jr. and by an Atomic Energy Commission contract with The Johns Hopkins University.)

METTLER, L. E. *See* GLASS, BENTLEY.

MEYER, HELEN UNGER. Modification of the Ultraviolet-Induced Rate of Autosomal Lethals by Nitrogen Pre-Treatment or Post-Treatment in *Drosophila*. *Department of Zoology, Indiana University, Bloomington, Ind., U.S.A.*

The influence of anoxia on ultraviolet mutagenesis in non-dividing embryonic germ cells (pole cells) of *Drosophila melanogaster* was studied in a series of experiments, in which part of the material was placed in an atmosphere of nitrogen for short periods, either as pre-treatment (lasting 5–12 min.) or as post-treatment (25 min.). A dose of 90 ergs/mm.^{2} of predominantly 2,537 Å was given simultaneously to all lots in each experiment, in all cases in air.

The rate of autosomal lethals (chromosome 2) was used to measure the genetic effects. Data from the various experiments in each series were summarized by the harmonic mean method designed by Muller (1941, 1952, and unpublished) for such purposes. The lethal rates given are these harmonically weighted mean

mutation rates, and the average number of tested chromosomes listed are "effective" means applying to each of the contrasted groups. The rate obtained from ultraviolet without nitrogen treatment is listed first in each of these comparisons.

Giving a nitrogen post-treatment as late as 15 to 20 min. after ultraviolet irradiation did not influence the lethal rates: 5.6 ± .9% as compared to 5.9 ± 1.1% for the group getting the delayed post-treatment, with a mean effective number of 1,363 tests in each lot. When post-treatment followed irradiation within about a minute, a somewhat lower frequency for the nitrogen post-treated group was obtained: 5.3 ± .8% versus 3.0 ± .6% (2,082 tests each). However, the opposite trend became apparent when the nitrogen treatment immediately preceded irradiation, leading to a significant increase of the lethal rate in the pretreated group: 4.5 ± .7% against 9.0 ± 1.6%, with 1171 effective tests for each.

We conclude that our earlier results (1952, 1953), showing increase of ultraviolet mutagenesis when anoxia was applied both before, during and after the irradiation, represented the predominant effect of the pre-treatment by anoxia.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the U.S. Atomic Energy Commission (Contract AT(11–1)–195).)

MICHAELIS, ARND. *See* RIEGER, RIGOMAR.

MICHIE, DONALD. The "Background Effect" on Phenotypic Variability. *Royal Veterinary College, University of London, London, England.*

The classical view of phenotypic variation interpreted the entire measurable variance in terms of the effects of genetic and environmental factors which vary from one member of the population to the next. Recent evidence is reviewed which suggests that different *uniform backgrounds,* whether genetic or environmental, result in different levels of phenotypic variability. Particular importance is attributed to uniform backgrounds which depart from the norm to which the given species has been adapted in its evolutionary past. Such backgrounds can be expected to act by *increasing* the phenotypic variance. Examples of such backgrounds are the presence in the genome of a mutant gene (Waddington, Nature, Lond. *150*: 563–565), the homozygous condition in a naturally outbreeding species (*see* Lerner, Genetic Homeostasis, Edinburgh, 1954), the genetic constitution of remote hybrids, and unaccustomed climatic, nutritional, or other environmental conditions. All these may be expected to weaken the "buffering" mechanisms of normal embryonic regulation, and thus make the individual members of the population more sensitive to the residual genetic and environmental factors which vary from organism to organism.

Possible implications of the background effect for evolutionary theory and selective breeding are suggested and discussed.

MICHIE, DONALD. *See* MCLAREN, ANNE.

MICKEY, GEORGE H. Polyploidy and Aneuploidy in *Drosophila melanogaster* Induced by Cold Shock Treatment. *Department of Zoology, Physiology and Entomology, Louisiana State University, Baton Rouge, La., U.S.A.*

First instar larvae of *Drosophila melanogaster* subjected to low temperature (about .0°C) for brief periods (2–4 hours) then permitted to recover for varying lengths of time (1–48 hours) exhibit both euploid and aneuploid cells in quash preparations of brain ganglia. Reduction in proportion of polyploid cells correlated with longer recovery periods indicates elimination of abnormal cells. Apparently only diploid cells persist to time of pupation. Single treatments produce tetraploid cells primarily; subsequent treatments after a recovery period result in some octoploid cells. Although strain differences in susceptibility to alteration by temperature shock obviously exist, extensive individual differences also are evident. No aberrations resulting from chromosomal breakage have been detected.

MIKAELSEN, KNUT. Comparisons between Cytological, Physiological and Genetic Effects of X- and Neutron-Irradiation in Barley. *Institute of Genetics and Plant Breeding, Vollebekk, Norway.*

A reported difference in radiobiological effects of X-rays and neutrons has been explained as the result of neutrons reacting more specifically with the cell nuclei while X-rays also exert their effects on cytoplasmatic cell constituents causing more physiological damage.

The differences between X-rays and neutrons will be discussed by comparing their effects on some cytological, physiological and genetic characteristics in barley.

Barley seeds were irradiated with X-rays and pile neutrons of different energies. The frequency of chromosome aberrations was studied and the results indicated that neutrons break chromosomes more seriously, revealing less restitution of broken ends, than X-rays. Seedling growth was measured, and the inhibition of growth seemed to be proportional to injured cell nuclei when analysed in metaphase, but not if analysed in anaphase.

Respiration was measured and the results indicated very similar effects for X-rays and neutrons. When the respiration was measured as oxygen uptake per any given number of seeds or dry weight unit, a significant reduction in respiration was found after both types of radiation. When the respiration rate was based on the amount of protein-N in the embryo, there was no difference between irradiated and non-irradiated seeds. Therefore, the respiration per unit active protoplasm was unchanged, and the respiration process itself was apparently not inhibited by the radiation.

Proteolytic activity in the endosperm and nitrogen transport from endosperm to embryo did not seem to be affected by the irradiations. Protein synthesis in the embryo, however, was inhibited in the irradiated seedlings. This reduced ability to synthesize protein was probably not due to any lack of necessary amino acids but rather to a reduction in nucleic acid content. This phenomenon may have some relation to the increase in dry matter content of the irradiated seedlings which was found. These experiments seem to indicate that there are no qualitative differences in the physiological effects of neutrons and X-rays and the quantitative differences are mainly due to differences in ion density of the two types of radiations.

Studies on mutation frequencies in barley seem to support this conclusion. No

difference in the mutation spectrum from the two types of radiation was demonstrated. The difference in biological effectiveness may be explained on the basis of the different ion density of X-rays and neutrons (both thermal and fast).

MILANI, RICCARDO. The Genetics of the House-fly (*Musca domestica*): New Mutants, Linkage Groups and Abnormal Segregations. *Istituto di Zoologia dell' Università, Pavia, Italy.*

Several mutant types, not described before, have been found in *Musca domestica.* Some of these have been found in unrelated populations; the genes involved appear to be fairly common, at least in some populations. The commonest mutants have poor penetrance and variable expressivity. A recessive mutant type drastically affecting legs and wings (fused tarsal segments and incompletely stretched, bubbled wings) provides a fairly high number of reversions to normal; the expressivity of the effect on the legs fades over a number of generations, while the effect on wings remains constant. Several mosaics with normal have been found.

An example of parallel mutation in *Drosophila* is provided by the mutant *countercoiled* (*ctc*).

The "good" mutants have been tested for linkage. So far, a single linkage group has been established. The recombination data show very low frequency of cross-overs when the heterozygous parent is the male, very high (approximating independence) when the heterozygous parent is the female. The low cross-over rates from heterozygous males vary according to the strain used. The mutants linked are: *brown body* (*bwb*); *divergent wings* (*dv*); *knockdown-resistance* (*kdr*).

Fully penetrant mutants can provide very variable segregation frequencies in different experiments: crosses involving palearctic and nearctic strains have provided very abnormal segregations in some F_2 families, where abnormal sex ratios are also common.

MILLER, DWIGHT D. Karyotype Variation in Several *Drosophila affinis* Subgroup Species. *Zoology Department, University of Nebraska, Lincoln, Neb., U.S.A.*

Chromosomes were studied at diakinesis in primary spermatocytes of *Drosophila affinis, algonquin, athabasca, azteca,* and *tolteca.* Some differences were observed with respect to published illustrations of chromosomes of these species (Sturtevant and Dobzhansky, 1936; Patterson and Stone, 1952). *D. athabasca* and *azteca* regularly had a rod-shaped fourth chromosome instead of the J-shaped fourth chromosome previously shown. Some intraspecific variation of karyotype was found in *D. affinis* and *D. athabasca.* A strain of *D. affinis* from New York had two pairs of microchromosomes instead of the single pair characteristic of this and the other species. These species were originally described as having a large J-shaped Y-chromosome (more than half as large as the X). Although this difference was confirmed in at least some strains of each species, the Y-chromosome was found to vary within *D. affinis* and *D. athabasca.* In *affinis,* a large Y-chromosome of the kind originally described was found in strains from Nebraska, New York, and Texas. However, a small J-shaped Y (less than half the size of the X) was observed in strains from Iowa, Kentucky, Minnesota, and Nebraska,

and one Nebraska strain had no Y-chromosome. In *D. athabasca*, western strains (Alaska, British Columbia, Wisconsin, and Wyoming) were found to have a large J-shaped Y, as did certain strains from Quebec. On the other hand, relatively small V- or J-shaped Y-chromosomes were encountered in strains from Michigan, Ontario, and Quebec. Moreover, strains from Michigan and New York showed evidence of a translocation between the Y and the fourth chromosome. Males of these strains had a large V-shaped Y (similar to the X), and this was associated not only with the X-chromosome but also with a single fourth chromosome, which must, therefore, represent a second X-chromosome. (Research supported by a grant from the National Science Foundation (U.S.A.).)

MILLER, JAMES R. Some Embryological Consequences of Maternal Fasting and Their Relation to the Phenocopy Concept. *Department of Genetics, McGill University, Montreal, Canada.*

The use of maternal fasting as a teratogenic agent in the mouse, first described by Runner, has been extended.

(1) The original "fasting effect," which Runner found, included anomalies of the skull, vertebrae, and ribs in 25 to 30% of the offspring of females of the strain 129 who had received a 24-hour fast on the ninth day of their pregnancies. The present work indicates that strain differences exist with respect to the time and degree of response to this treatment. These gross differences may be indicative of more subtle differences, e.g., in the time and rate of somite differentiation and/or placental development and attachment.

(2) The frequency of the cortisone-induced cleft palate in strain C57BL/6, described by Fraser and his students, can be increased considerably by a maternal fast of 24 hours, 48 hours prior to the first cortisone injection.

(3) The frequency of the sporadically occurring eye anomalies which occur in strain C57 is significantly increased in the offspring of females of the BL/6 subline who receive a 24-hour fast on the ninth day of pregnancy.

These findings will be discussed in relation to the two major genetic mechanisms which Goldschmidt postulates underlie the production of phenocopies.

MILLER, R. A. *See* WALKER, G. W. R.

MILLER, WILMER J. Subtypes in Cattle Blood Typing. *School of Veterinary Medicine, University of California, Davis, Calif., U.S.A.*

In genetic systems of blood groups involving multiple alleles, it is a general rule that many of the diagnostic reagents (antibodies) used in the differentiation of the agglutinogens or phenogroups (products of the individual alleles) exhibit so-called "subtyping" relationships. The classical example involves the subtypes A_1 and A_2 of blood factor A of man, the first of which is characterized by reaction with both anti-A and anti-A_1 reagents, whereas the second reacts only with anti-A. Many subtyping relationships of the same asymmetrical order as A_1 and A_2 have

been noted with reagents reacting in the A-H, B, C, F-V, M and S-U systems of cattle. Far less attention has been given to the non-linear partitions among subtypes. A cogent example in man is that involving the so-called anti-C agglutinins which cross react with the human agglutinogens A and B but not O. Both anti-A and anti-B exhibit linear asymmetrical relationships with respect to anti-C but not with respect to each other. It is now established that many of the reagents reacting with phenogroups within such systems as B and S-U of cattle exhibit both linear and non-linear subtyping relationships. Intergradations of subtypes with other specificities, often regarded as serologically independent even though belonging to the same system of blood groups, demonstrate the need to emphasize the phenogroups rather than the individual blood factors which characterize them. Examples of cross reactions involving both linear and non-linear relationships are presented and discussed.

MITCHELL, H. K. *See* GLASSMAN, E.

MIZUSHIMA, USABURO, and KIYOSHI KATSUO. Elimination of Self-Incompatibility in the Common Cabbage, *Brassica oleracea* L., by Means of Substitution of Nucleus. *Institute of Plant Breeding, Faculty of Agriculture, Tohoku University, Sendai, Japan.*

A self-compatible strain of artificial amphidiploid(BC, $n = 17$) obtained between two self-incompatible species, *Brassica nigra*(B, $n = 8$) and *B. oleracea*(C, $n = 9$), was backcrossed to a self-incompatible common cabbage plant, the latter being continued vegetatively and used always as the pollen parent. Among the aneuploid offsprings there were found three plants that seemed to have diploid cabbage genomes in the cytoplasm of the amphidipoid which is of *nigra* origin. They resembled closely the recurrent cabbage parent in their morphology, showing normal head formation. The microsporogenesis carried out in them was quite regular with the formation of nine bivalents at MI. They were poorly fertile, but the authors were successful in continuing them by selfing. After six years of successive selfing, however, they became highly fertile and self-compatible, presenting more than 70% self-fertility. Unlike the ordinary cabbage, they showed no noticeable weakening of vigor. They always give self-compatible offsprings in crosses with the original, self-incompatible cabbage parent so far as they are used as the female parent, but give only self-incompatible ones in the reciprocal crosses, showing a clear cytoplasmic action to nullify the effect of the incompatibility gene or genes of the male parent.

MOHLER, J. D. Material for the Study of Multifactorial Gene Action. *Department of Zoology, Oregon State College, Corvallis, Ore., U.S.A.*

The concept of multifactorial inheritance is well established in genetics. Yet a major void exists. Beyond the statistically easy, but often experimentally unrealized additive concept, few general or specific ideas about the nature of gene action in such systems are current. A program which approached this problem

from a phenogenetic point of view might provide some basic clues. For this purpose work has been started on a number of "crossveinless-like" (*cvl*) strains of *Drosophila melanogaster* which were selected from a variety of wild-type sources.

The present efforts are upon the genetics of some of these lines. Progress to date shows that the inheritance is not strictly multigenic, but that in each line some major switch gene is apparently present. For each, however, penetrance varies as the residual genotype is varied. Two sublines, derived from a common ancestor, with a sexlinked *cvl* have a remarkable difference in the modifier(s) in the second chromosome. In one case this chromosome is definitely suppressing; yet the penetrance in the two stocks appears to be the same. In addition to lines with a sex-linked factor another strain has a third chromosome *cvl* gene. The relationships of these switch genes to loci such as *cv* of the X and *cv-c* of the third chromosome are being investigated. That all tested chromosomes in each line do have some effect on penetrance is suggestive of a multifactorial nature of the modifying genes.

Though the trait in these cases is not determined by individually unrecognizable multifactorial differences from wild type, the lines may still be expected to be valuable tools. Indeed, only of this kind of material could one ask, how specific are the multiple modifiers of mutants at complementary loci?

MOLL, R. H. *See* ROBINSON, H. F.

MONTALENTI G., and TADINI G. VITAGLIANO. Multiple Sexual Genotypes in a Gonochoristic Species *Asellus aquaticus*. *Istituto di Genetica, Università di Napoli, Naples, Italy.*

Various sibships of wild *Asellus aquaticus* show a significant variation in sex ratio. Besides a number of families in which the sex ratio among sibs is 50%, others are found in which the ratio is shifted considerably and significantly toward the male (arrhenogeny) or the female sex (thelygeny). The sex ratio of the total progeny of all pairs approaches the normal (50%). This gonochoristic species has a wide range of variation of sexual genotypes, going from equality to almost complete monogeny. The tendency to arrhenogeny or to thelygeny is hereditary. The intensity of the character can be increased by selection, but in the crosses between the extremes of the same tendency a high degree of sterility results. This sterility is entirely independent of consanguinity. On the contrary the crosses between arrhenogenic and thelygenic individuals show a high heterosis. The genetic mechanism underlying such a situation is probably a polygenic sex determination with a great deal of recombination occurring at each generation. The evolutionary meaning of this condition is discussed.

MONTALENTI, G. *See* SINISCALCO, MARCELLO, *et al.*

MOORE, C. H. *See* BELL, A. E.

MOORE, RAYMOND J., and GERALD A. MULLIGAN. A Study of Natural Hybridization between *Carduus acanthoides* and *Carduus nutans*. *Botany & Plant Pathology Laboratory, Science Service, Ottawa, Canada.*

Natural hybridization between the introduced thistles *Carduus acanthoides* L. and *C. nutans* L. was noted in Grey County, Ontario, in 1951. A survey was made in 1952 and the population of one field was studied in detail as to morphology, fertility and chromosome number. The morphological variation was evaluated by means of a hybrid index. The chromosome numbers of the species are: *C. acanthoides*, $2n = 22$; *C. nutans*, $2n = 16$. All the intervening numbers occur in the hybrids, the number being correlated with the hybrid index. Among seedlings raised from seed of hybrid plants it was found that those with the chromosome number 22 were more frequent than those with other numbers. It was suggested that gametic selection may be acting to favour production of hybrids approaching more closely to *C. acanthoides* than to *C. nutans*. Grey Co. was revisited in 1957 and the present distribution of species and hybrids was recorded. Hybrid populations of 26 fields were evaluated in terms of the hybrid index scale. Hybrids were common in an area of 320 square miles in southern Grey Co., an area that seems to have been first colonized by *C. nutans*. Hybridization occurs usually on a field basis: individual fields are sites of primary hybridization, both parents and hybrids being present, or fields may contain only one parent and hybrids. In an area of approximately 18 square miles around the town of Flesherton, the parents are now rare, but hybrids are extremely common. These approach so closely to *C. acanthoides* that only careful, experienced observation would detect their hybrid nature. It is believed that hybridization first occurred in this area. The rapid dominance of the *acanthoides* phenotype supports the view that selective factors favour segregation toward this species. Fertility appeared to be higher than noted on the previous survey. The Flesherton *acanthoides*-type hybrids have spread, probably in road gravel, to a point 21 miles west of Flesherton where a large stand has become established in the past 2 to 3 years.

MORGAN, WALTER C., and RAYMOND GREB. Evolutionary Import of a Newly Discovered Mutation Affecting the Genital System in Poultry. *South Dakota State College, Brookings, S.D., U.S.A.*

Schematic patterns which outline the biological evolution of species have been theorized and expanded upon, primarily since Darwin's publication of *The Origin of Species*. Studies of fossil remains have supported evolutionary hypotheses concerning gross structural alterations. The detailed comparison of body systems and accompanying homologies, as based largely upon embryologic similarities and differences, has given rise to the phylogenetic sequence within the animal kingdom which is used as a basis for most comparative anatomy courses. However, there is a paucity of direct evidence concerning visceral changes which alter internal body systems. The purpose of this report is to consider the evolutionary potential of a

mutation which grossly alters one of these body systems: the reproductive system in hens.

In many of the vertebrate species the reproductive tract consists of bilateral gonads and bilateral ducts servicing these gonads. However, in the domestic hen *Gallus gallus* there are reproductive organs (ovary and oviduct) only on the left side. Both sexes have bilateral structures during early embryological development, but there is, normally, a persistency of only the structures on the left side in females.

A specific line of Rhode Island Reds at the South Dakota Experiment Station has had a very high incidence of bilateral oviducts, >75%. This inherited condition could, conceivably, be regarded as a beneficial mutation because it provides the females with twice the normal egg-forming potential following expulsion of ova from the ovary. No effect has been observed in the male. It is possible that the mutation may be located on the disputable female sex chromosome, inasmuch as in birds the female is the heterogametic sex.

Two other points of importance are: (1) no right ovary has been observed (which is not too surprising owing to their separate embryonic origins), and (2) the presence of two oviducts would suggest a reverse-mutation, a return to the primitive state. New evidence to be included in this report will include additional autopsy data, findings from histologic sections, and pedigree charts showing the relationship of mutant females.

MORIWAKI, DAIGORO, and Y. NAKAJIMA. Maternal Effects on Heterosis owing to an Inversion in *Drosophila ananassa*. *Department of Biology, Tokyo Metropolitan University, Tokyo, Japan.*

In *Drosophila ananassa* an inversion, In II L, in the left arm of the second chromosome has long been known, two different types of gene arrangements, A and B, being distinguished. Experiments with these types, made in population cages in our laboratory, showed that a balanced polymorphism has been established by heterosis. In order to analyse the adaptive advantage of the AB heterozygotes, comparisons were made among the four karyotypes, AA, BB, AB, and BA, with respect to the physiological traits of the bearers. The results showed that marked heterosis could be expressed in the heterozygotes in the rate of development of larvae and pupae at both 25°C and 19°C, and in viability under unfavorable conditions at 25°C.

On the other hand, maternal effects on the heterozygotes, AB and BA, in the rate of development were remarkable at 25°C. These effects, seen in the F_1 heterozygotes, seem to be inherited in the next generation.

Whether the effects would be inherited through subsequent generations is still unknown, and further experiments are going on.

MORLEY, F. H. W. Sub-speciation in *Trifolium subterraneum* L. *C.S.I.R.O. Division of Plant Industry, Canberra, Australia.*

Trifolium subterraneum L. is an annual, self-fertilizing legume, derived from the Mediterranean region, now widespread in parts of Australia. The discovery of sterility in some F_1s led to an investigation of the genetic structure of this species.

Twenty "Australian" strains could be divided, on the basis of fertility of F_1s and later generations, into one set of sixteen strains (Group A), the two others, B and C, each containing only two strains.

Fertile F_1s were obtained in crosses of the Australian Group A with 6 of 11 strains from the Iberian peninsula, 4 of 10 strains from Morocco, 2 of 6 from Algeria, 2 of 2 from Turkey, and 1 of 4 from Greece. Other crosses with A strains produced infertile or non-viable F_1s, as did crosses of A strains with single strains from each of Israel, Cyprus and the Canary Islands. Of 21 F_1s from crosses among strains incompatible with Group A, the majority from different regions, all were highly sterile or non-viable. Thus there appear to be *numerous sub-species in the original habitat*. The Australian sample differs from the Mediterranean sample in containing such a high proportion of strains in Group A, and representatives of only three affinity groups. This suggests that these *introductions to Australia came from a restricted portion of the original habitat.*

There are no indications that affinity groups other than A are widespread, as would be expected if the distribution of A were determined by migration. The pattern of distribution observed suggests that *subspecific differentiation occurred on many independent occasions, and in a variety of locations, rather than at a centre of variation.*

MORPURGO, GIORGIO, and GIUSEPPE SERMONTI. Genetic Instability in Diploid Strains of *Penicillium chrysogenum*. *Istituto Superiore di Sanita, Rome, Italy.*

A conidial suspension of the green diploid strain of *Penicillium chrysogenum* $\frac{+\ +\ w\ cy}{y\ py\ +\ +}$ was treated with nitrogen mustard to give about 0.5% survival. Treated conidia were plated on complete medium: colonies arising from these conidia show a very increased percentage of white and yellow, as sectors or whole colonies (30 to 40 per cent) if compared with segregation of the control (1%−). One-third of the green conidia from the colonies showing yellow or white sectors, plated without further treatment on complete medium, show a very high rate of sectoring (almost 100%). From the few colonies which do not give sectors, stable subclones in which the markers showing a high segregation rate were still present in heterozygous condition were readily obtained. The tendency to give sectors may be maintained for an indefinite number of generations by plating green conidia. Nine unstable clones from different colonies have been isolated so far. One other unstable clone was derived from a mosaic colony which had formed spontaneously. Each clone always gives sectors of the same type: 4 clones give yellow prototroph sectors; 1 yellow cysteine; 1 white prototroph; 1 white cysteine; and 2 give two types of sectors, namely white cysteine and yellow pyridoxine. In four strains in which we have investigated, after treatment with N-mustard or U.V., we were able to find markers which do not normally appear in the sectors.

Sectoring is completely abolished if conidia of unstable lines are plated on complete medium + 0.8% of $MnCl_2$. Nevertheless conidia of such colonies grown on C.M. + $MnCl_2$, plated on C.M. without $MnCl_2$, again give sectors. Salts of Ca^{++}, Co^{++}, Cu^{++}, Fe^{++}, Mg^{++}, Ni^{++}, do not produce this effect. $MnCl_2$ also diminishes the percentage of segregants appearing as sectors or whole colonies after treatment with N-mustard.

The authors discuss the possible explanations of such genetic instability.

MORPURGO, GIORGIO. *See* SERMONTI, GIUSEPPE.

MORRIS, ROSALIND. *See* LIMA-DE-FARIA, A.

MORRISON, JOHN H. The Effect of Monoiodoacetic Acid on Cell Division. *Michigan State University, East Lansing, Mich., U.S.A.*

Treatment of root tips of *Pisum* with monoiodoacetic acid inhibits the chromosome movement cycle during all stages of the mitotic process in meristem cells without apparent alteration of the morphological cycle of the chromosomes. Iodoacetic acid prevents kinetochore cleavage with the result that chromosomes in metaphase configurations undergo telomorphic transformation *in situ*. The study and measurement of the cytological effects of iodoacetic acid show that the time required to obtain complete inhibition of chromosome movement of a given mitotic stage is dependent on the molar concentration of iodoacetic used. The fact that iodoacetic acid is known to inhibit the triosephosphate dehydrogenase system which is one of several pacemaker reactions in anaerobic glycolysis, the results of the iodoacetic acid reaction suggest that there exists a relationship between the energy-yielding reactions of glycolysis and the chromosome movement cycle during the mitotic process.

MORTON, N. E., and C. S. CHUNG. Formal Genetics of Muscular Dystrophy. *Department of Medical Genetics, University of Wisconsin, Madison, Wis., U.S.A.*

Genetic classes of muscular dystrophy delimited by pedigree information and discriminant functions (Chung and Morton, X Genetics Congress) were analysed by maximum likelihood scores. Group I is generally attributable to an autosomal dominant gene of complete penetrance in individuals who survive to the age of onset. The clinical type is facio-scapulo-humeral. Isolated cases of group II are mostly true sporadics, of undetermined etiology, while familial cases are consistent with an autosomal recessive gene or genes with complete penetrance above the age of onset. The segregation analysis confirms the phenotypic evidence that about 40% of group II cases are sporadic, this proportion being homogeneous among sources of data. In both isolated and familial cases, the clinical type is usually limb girdle, sometimes Duchenne. Group III cases are sex-linked and Duchenne in type, with complete penetrance. Cases of group IV, classified by a discriminant function into probably of genetic type II or probably of genetic type III, are found to have the distribution expected on this hypothesis.

When the sex-linked cases are analysed with respect to the proportions of sporadics and of affected uncles, excellent agreement is obtained with frequencies predicted on the assumption of equal mutation rates in egg and sperm. The assertion of Haldane, that there is deficiency of sporadic cases and therefore evidence for higher mutation rates in males than in females, is based upon an inappropriate formula, and there is no evidence for his claim even in the data which he examined.

The proportions of sporadics and of affected uncles are homogeneous among sources. Estimates of incidences, gene frequencies, fertilities, mutation rates, and genetic risks by several independent methods will be given.

MORTON, N. E. *See* CHUNG, C. S.

MOSSIGE, JEANNE. Translocations and Recessive Sex-Linked Lethals Induced in *D. melanogaster* by Ingested Radiophosphorus. *Norsk Hydro's Institute for Cancer Research, Norway.*

The findings of Oftedal and Mossige that incorporation of P^{32} in sperm followed the same curve as the mutation rate for sex-linked recessive lethals in daily broods suggested the possibility that some of the mutations might be due to the disintegration of P^{32} incorporated in the genetic material. In an attempt to elucidate this question the day-to-day mutation curve for II–III translocations was determined.

After an acute dose of radiation to newly eclosed males, the daily sensitivity pattern for translocations and recessive lethals has been determined. If, after ingestion of P^{32}, an appreciable number of mutations is due to disintegrations in the genetic material, and if these disintegrations are able to produce breaks leading to translocations as well as to sex-linked lethals, translocations should be found among the progeny of days 9 and later as these could be induced by disintegration in postmeiotic sperm. No such translocations have been found.

MOTA, MIGUEL. Karyokinesis without Cytokinesis in the Grasshopper. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*, and *Estação Agronómica Nacional, Sacavem, Portugal.*

Preparations of living grasshopper spermatocytes of the species *Melanoplus differentialis* and *Chortophaga viridifasciata* (De Geer) were made to provide material for an experimental study of the anaphase movement. In these preparations many cells were found where cytokinesis had not followed karyokinesis, resulting in binucleate cells from both primary and secondary spermatocytes. In some of these cells a clearly visible ring of jelly-like substance, brighter under phase microscope than the surrounding cytoplasm, could be seen constricting the middle of the bunch of mitochondria that outlines the spindle remnants, very much in the same way the cleavage furrow does in a normal cytokinesis. The ring did not last more than fifteen minutes under observation, breaking and fading away after that period of time or earlier. The origin of this aberration is unknown.

The finding of such a ring gives support to the theories that explain cell cleavage assuming the existence of a contracting ring located on or immediately under the cell membrane at the equator. It seems clear that in these cases the ring contracted but separated from the cell membrane.

Motulsky, Arno G. Joseph Adams (1756–1818): The Forgotten Founder of Medical Genetics. *Department of Medicine, University of Washington, Seattle, Wash., U.S.A.*, and *Galton Laboratory, University College, London, England.*

A British physician-apothecary, Joseph Adams, published a book in 1814 entitled *A Treatise on the Supposed Hereditary Properties of Diseases Based on Clinical Observation.* The identical book was reissued in 1815 under the title *A Philosophical Treatise on the Hereditary Peculiarities of the Human Race.* The author showed remarkable insight into some principles of human genetics still applicable today. As far as can be ascertained, little attention was given to his findings.

The stated aim for the book was to furnish a guide for heredity counselling. Adams made the following observations. (1) Clear distinction between congenital, familial ("recessive") and hereditary ("dominant") conditions and their patterns of inheritance; (2) some of the characteristics of familial (recessive) diseases were noted, i.e., mating between relatives would increase the disease frequency. (3) Hereditary diseases may manifest at various age periods and not necessarily at birth. (4) Hereditary susceptibilities exist which need external precipitating factors to make illness apparent. In terms of risk for offspring, it is immaterial whether clinical disease is present or not. (5) Genetic illness may be treatable especially if precipitating factors may be removed. (6) Intrafamilial correlation of age of onset of hereditary diseases exists and is clinically useful in genetic prognosis. (7) He hinted at genetic heterogeneity of clinically identical disease. (8) Increased hereditary disease frequency in isolated districts may be caused by inbreeding. (9) He logically predicted mutations by stating that genetic disease would disappear due to early death of the affected were it not for new origin of the condition in offspring of parents free from it. Adams' attitude to practical eugenic measures was critical and again quite modern. He concluded by calling for the establishment of hereditary disease registers for further study.

Joseph Adams' biography and the failure of medical scientists to note his observations will be discussed against the background of early nineteenth century science and medicine.

Moutschen, Jean. Action combinée du Myleran et des rayons X sur les graines de *Vicia faba* L. *Institut de Morphologie Végétale, Laboratoire de Cytogénétique, Université de Liège, Liège, Belgique.*

Le Myleran, 1–4 bis-(méthyl-sulfonyloxy) butane, utilisé sur des graines de *Vicia faba* avant et après irradiation par les rayons X, modifie considérablement les effets de ceux-ci sur les chromosomes. Un temps d'action de 20 minutes (20 mg./100 cc.) est suffisant pour inhiber en grande partie la genèse des ponts chromosomiques apparaissant normalement après irradiation. D'autre part, les cassures chromosomiques dues au Myleran et aux irradiations agissant simultanément s'additionnent. Le traitement par le Myleran après irradiation produit encore des modifications dans les effets des rayons variant avec le moment auquel la substance est donnée. Le Myleran donné 16 heures après l'irradiation relève fortement le taux de ponts chromosomiques mais celui-ci n'atteint cependant jamais le taux que l'on observe après les rayons X seuls.

Les modifications de la répartition des cassures chromosomiques dues à l'action

conjuguée des deux agents ont été étudiées et l'intérêt pour la cytogénétique de l'action de ces agents en combinaison a été souligné.

MOUTSCHEN, JEAN. *See* MOUTSCHEN-DAHMEN, MADELEINE.

MOUTSCHEN-DAHMEN, MADELEINE, et JEAN MOUTSCHEN. Le maintien en première génération des lésions chromosomiques dues à la 8-éthoxycaféine chez *Vicia faba* L. *Institut de Morphologie Végétale, Laboratoire de Cytogénétique, Université de Liège, Liège, Belgique.*

Les lésions classiques dues à la 8-éthoxycaféine ont été suivies dans 30 plantes de *Vicia faba* L. provenant de graines traitées. De ces 30 individus, 90 ont été obtenus en première génération. De nombreuses lésions chromosomiques ont été retrouvées dans les méristèmes radiculaires de ceux-ci et leur évolution a été également suivie. La répartition dans le génome de ces lésions chromosomiques relevées en première génération a été comparée à la répartition des lésions observées chez les parents; la spécificité de localisation des cassures dues au toxique se maintient.

Il est étonnant qu'au grand nombre d'aberrations chromosomiques spécifiques relevées ne corresponde qu'un nombre restreint d'anomalies morphologiques.

MUKAI, TERUMI, and ALLAN B. BURDICK. Single Gene Heterosis Associated with a Second Chromosome Recessive Lethal in *Drosophila melanogaster. Department of Biological Sciences, Purdue University, Lafayette, Indiana, U.S.A.*

A clear demonstration of single gene heterosis for viability associated with a second chromosome recessive lethal is described in this report.

In a case of superviability of heterozygotes, the proof of single gene heterosis must rest on the elimination of the contribution of (*a*) the genetic background, and (*b*) specific interaction between the genetic background and the gene in question towards the observed superviability. The former can be achieved by tracing the frequency of lethal heterozygotes under random mating generation after generation because the genetic background becomes equalized for the two kinds of flies ($l/+$ and $+/+$) by crossing over and random mating if the lethal is not contained within an inverted segment. The latter can be achieved by testing the degree of heterosis in different genetic backgrounds.

The lethal gene is located at about 54 on the second chromosome and is not associated with crossing-over inhibition. Four populations were established, the frequencies of lethal heterozygotes in the starting generation being 1 in Populations 1 and 2, and 0.05 in Populations 3 and 4. The genetic backgrounds of Population 1 and 3 were the same and those of Populations 2 and 4 were the same. The populations were established and tested at different times but were propagated under the same schedule and density. The four populations reached the same equilibrium point where the frequency of lethal heterozygotes is about 0.42, and this has been maintained for about 300 generations.

The experimental results indicate superviability of lethal heterozygotes and from other experimental results, the cause of superviability is attributable to higher fecundity of lethal heterozygote females and/or better mating ability of lethal heterozygote males.

In conclusion, it can be said that the superviability of these lethal heterozygotes is due to single gene heterosis.

MUKAI, TERUMI. *See* BURDICK, ALLAN B.

MUKHERJEE, BARID B. *See* VICKERY, ROBERT K.

MULLIGAN, GERALD A. *See* MOORE, RAYMOND J.

MUNDAY, ANN. *See* FRANKEL, O. H.

MUNKRES, K. D., V. W. WOODWARD, and V. SUYAMA. Metabolic Analyses of Certain Pyrimidine-Requiring Mutants of *Neurospora*. *Kansas State College, Manhattan, Kan., U.S.A.*

The evidence that pyrimidines are metabolized by dual pathways in *Neurospora*, as in bacteria and mammals, is threefold: (1) growth studies, (2) accumulation products, and (3) enzyme activity. The significance of the dual pathways in terms of genetic-metabolic relations will be discussed. That a reductive pathway for uracil metabolism exists in *Neurospora* was first suggested by positive growth responses of the pyrimidine-requiring mutant, pyr-1 (263), to dihydrouracil (DHU) and B-ureidopropionic acid (B-UP). We now have other mutants, 1g, pyr-5, and KS-12, that respond only to DHU. Still another class of mutants (pyr-3 and its pseudoalleles) respond only to uracil. No pyrimidine mutant responds to B-alanine. Analyses of culture filtrates of 73a, pyr-1, pyr-3, pyr-5, KS-12 and 1g, when grown on uracil, reveal DHU and B-UP, whereas minimal cultural filtrates of 73a do not. Pyr-5 and KS-12 are unique in that B-UP accumulates in much higher concentrations. Finally, enzyme activity for the hydrolysis of DHU to B-UP in pyr-1, pyr-3, and 73a has been detected. Tuttle and Mitchell (personal communication) have observed enzymic conversions of ureidosuccinic acid (US) through to uridine in the orotic acid pathway, as well as the accumulation of dihydroorotic acid (DHO) and US in pyr-1. We have corroborated the latter and, in addition, have found that pyr-5 and KS-12 accumulate US and either DHO or 5-carboxymethylhydantoin or both. (This research was supported by a research grant from the National Institutes of Health, RG-4561 (C2). Contract No. 617, Kansas Agricultural Experiment Station.)

MUNKRES, K. *See* WOODWARD, VAL W.

MURAKAMI, UJIHIRO. On the Pathogenesis of Congenital Malformations of the Central Nervous System: A Study of Phenocopies of the Mouse. *Research Institute of Environmental Medicine, Nagoya University, Chikusa-Ku, Japan.*

Mice were injected with 0.1 cc. of 1% trypan blue between the sixth and ninth day of pregnancy and the uterine contents were examined on the thirteenth day. In the group treated on the eighth day, pseudencephalies were seen more frequently than in groups treated on other days. Mothers exposed to hypoxia on the eighth day of pregnancy produced many embryos with curvatures of the spinal cord, plications of the brain, and hydrocephalus. In both experiments, other kinds of abnormalities corresponding to distended central nervous system as described by Snell *et al.* were seen—especially in embryos dying during the tenth to twelfth day of embryonic life. These distensions of the central nervous system were often associated with enlargement of the pericardium.

When such distensions of the central nervous system were examined microscopically, proliferation of ependymal elements with abundant mitoses often accompanied by excess cerebrospinal fluid was found to underlie overgrowth and distortions of the central nervous system. Numerous transitional forms of central nervous system malformation were observed, varying from uncomplicated distension of the central nervous system to pseudencephaly, brain hernia, brain plication, spina bifida, or combinations of these defects.

In young embryos in which the choroid plexus is not yet formed, cerebrospinal fluid is produced by ependymal cells and presumably when ependymal cells proliferate excessively their function of producing cerebrospinal fluid is also accelerated. Sometimes small elevations of ependymal elements were seen in the brain of an embryo with hydrocephalus. I suggest that hydrocephalus, pseudencephaly, brain hernia, and spina bifida, originating in embryonic life, have their basis in the proliferation of ependymal elements which represents a non-specific reaction to diverse agents including X-radiation, P^{32}, and HVJ-virus infection.

MURAMATSU, M. *See* MATSUMURA, S.

MURRAY, MERRITT J. Evolution in the Genus *Mentha*. *A. M. Todd Company, Kalamazoo, Mich., U.S.A.*

Taxonomists have long believed that the genus *Mentha* consists of five fertile pure species and numerous sterile F_1 hybrid combinations between these species. For example, fertile wild spearmint *Mentha spicata* L. ($2n = 48$) is considered an F_1 hybrid between *Mentha rotundifolia* L. ($2n = 24$) and *M. longifolia* (L.) Huds. ($2n = 24$). The pioneer cytogenetic work of Mabel L. Ruttle (Mrs. B. R. Nebel), 1929–38, has shown that the basic fertile species hybridize freely to form fertile *M. Niliaca* Jacq. em. Briq. with 24 chromosomes. We have confirmed her observations and found that the F_1 hybrids are Niliaca-like in appear-

ance, but differ from sterile *M. Niliaca* ($2n = 36$) in oil composition. Neither the diploid nor the double-diploid hybrids resemble *M. spicata* in appearance. In oil chemistry, the basic species and their F_1 hybrids have the ketones piperitone oxide and piperitenone oxide whereas the very polymorphic species *M. spicata* may have these same ketones or carvone and dihydrocarvone or menthone and pulegone. While the evolution of the ketone differences is due to the mutation and interaction of two dominant genes, the exact parentage of *M. spicata* is uncertain. On the other hand, sterile peppermint *M. piperita* L. ($2n = 72$) is synthesized by hybridizing water mint *M. aquatica* L. ($2n = 96$) to menthone and pulegone strains of *M. spicata* ($2n = 48$). Herbarium material will be used to illustrate the species and hybrids.

MURTY, B. RADHAKRISHNA, and N. R. BHAT. Male Sterility in *Nicotiana tabacum*. *Central Tobacco Research Institute, Government of India, Ministry of Food and Agriculture, Rajahmundry, India.*

The nature of action of the duplicate factors controlling male sterility in a spontaneous mutant of *Nicotiana tabacum* is described. Male sterility was recessive to normal cytological studies of the breakdown of microsporogenesis in the mutant, and the sterile segregants from crosses with other unrelated types showed that in addition to the deformation of the anthers, the breakdown at several stages started from the differentiation of tapetum while megasporogenesis was inferentially normal.

Studies of F_3 progenies from partial sterile and normal segregants of F_2's revealed that one of the duplicate genes is responsible for the restriction of the sporogenous tissue by deforming the anther. The other gene operated only in succession to the first and controlled the tapetal differentiation and other meiotic irregularities following the abnormal development of tapetum. The partially sterile segregants were never true breeding and segregated into 1 normal: 2 partial: 1 completely sterile, indicating that they are heterozygous for one gene and homozygous recessive for the other.

The regular association of the characteristic reduction in size and width of corolla tube with male sterility in all families indicated the pleiotropic effect of the mutant genes.

It is inferred that the absence so far of reports of male sterility in the putative species of *tabacum* is probably due to the elimination of deleterious genes in the diploid species as suggested by Clausen and Cameron (1952) or due to the complementary duplicate action of the male sterility genes in successive stages.

NAKAJIMA, Y. *See* MORIWAKI, DAIGORO.

NAKAO, YOSHIO. Further Study on Specificity of Interactions between the Individual Gene Locus and the Structure of Chemical Mutagens. *Biological Institute, Kyoto Prefectural Medical College, Kyoto, Japan.*

Using the egg colour mutants of the silkworm we have discovered a specific relationship between individual gene locus and two related agents of the mustard groups,

nitromine and N.M.-alanine, (Nakao, Tazima and Sakurai, Z.i.A.V., in press). Further, relative frequencies of the same mutants induced by N.M.-glycine (N-bis-β-chloroethyl-glycine) and N.M.-alanine-amide (N-bis-β-chloroethyl-alanine-amide) have been tested. In the silkworm, *pe* and *re* are located on chromosome V at 0.0 and 31.7, and both of them control the colour of the egg (and also colour of the eye). Whereas the normal colour is black, *pe/pe* eggs are white and *re/re* eggs red. Eggs homozygous for both *pe* and *re* are also white. We injected 0.05 ml. of either solution of N.M.-glycine (1/30%) or N.M.-alanine-amide (1/50 %) into the abdomen of the wild male moths which were then mated to double recessive *pere/pere* female moths. Eggs produced from these matings were examined for their colour and the mutation rates were estimated by the ratio of uncoverings at the marked loci to the total pigmented eggs.

Analysis of the results has revealed that the ratio of the mutation rates at *pe*-locus to those at the *re*-locus varies considerably according to the mutagens injected. When N.M.-glycine was used, the ratios were 1.06 and 1.31; when N.M.-alanine-amide was injected, the ratio was 0.07.

NATARAJAN, A. T. *See* SWAMINATHAN, M. S.

NAVASHIN, MICHAEL S. Cell Size and Development of Characters. *Botanical Institute of the Academy of Sciences of the USSR, Leningrad, U.S.S.R.*

It is a well-known fact that tetraploid individuals do not represent enlarged copies of their diploid progenitors although they differ from the latter merely in possessing reduplicated genomes, i.e., in a purely quantitative way.

As shown by numerous investigations the primary effect of tetraploidy consists in doubling the volume of the protoplast of the meristematic cell. In the course of ontogenesis, however, additional features become manifest, both in specialized cells and in organs. It was thus found that, although all the cells of the tetraploid plant are on the whole larger than those of the diploid, the increase is different in different tissues, and what is still more striking, the organs of the tetraploid acquire morphological peculiarities of their own. Among these the most important characteristic is an altered width/length ratio which is markedly increased in tetraploids.

The author has focused his attention upon the fact that the volume of organs (especially of those which possess determinate growth, e.g., seeds) is in tetraploids less than twice that found in diploids, as could be expected from the doubled cell size. Measurements have shown that the volume of organs in tetraploids is 1.6 times greater than in diploids, this ratio recurring with remarkable constancy. Starting with the observation that the value 1.6 practically coincides with the ratio $\frac{2}{\sqrt[3]{2}}$, the author advanced a hypothesis according to which the morphological peculiarities of the tetraploids depend basically on a decrease in relative surface of their meristematic cells which is obviously proportional to $\frac{1}{\sqrt[3]{2}}$ and represents an unavoidable result of an increase in absolute dimensions. If we assume that the decrease in the rate of cell division known to be connected with tetraploidy is proportional to the decrease in relative cell surface, the peculiarities

of tetraploid individuals will receive a natural explanation. Namely, an increase 1.6 times (instead of 2.0 times) will be understood as a consequence of doubling cell volume combined with a corresponding decrease in their number $\sqrt[3]{2}$ times; which thus should result in an $\frac{2}{\sqrt[3]{2}} = 1.61$ ultimate increment. An increase in the relative width of elongated organs would also appear to be a simple geometrical consequence of reduced number of cells along the axis of predominating propagation of cells, the latter at the same time possessing greater dimensions.

The author's hypothesis was verified on pappus bristles of diploid, triploid and tetraploid individuals of Russian dandelion (*Taraxacum kok-saghyz* Rodin). The number of cells composing these structures proved to be inversely proportional to cubic radicals of the corresponding cell volumes (i.e., 2, 3 and 4). Several suggestions are made by the writer as regards physiological importance of variation in cell size. Cell size is considered by him to be a primary factor of initial differentiation; quantitative variation in cellular elements may thus result in qualitative characteristics of the organism.

NAYLOR, J. K. *See* STICK, H. F.

NELSON, OLIVER E., Jr. Intercrosses among Alleles at the Waxy Locus in Maize. *Botany and Plant Pathology Department, Purdue University, Lafayette, Indiana, U.S.A.*

The *Wx/wx* locus in maize governs the type of starch produced in both the microspore and the endosperm. *Wx* starch is a mixture of amylose and amylopectin and stains blue-black with a dilute solution of KI and I_2, while *wx* starch lacks amylose and stains reddish-brown with the iodine solution. The starch type of a microspore is conditioned by its own genetic constitution and not by that of the plant producing it. This makes it possible to use the large number of microspores produced by a single plant as a population for genetic investigation, thus obtaining a resolution far higher than is usually possible with higher organisms.

All possible intercrosses have been made between six independently occurring mutations at the *Wx/wx* locus. Pollen samples were collected shortly before pollen shedding from the parental *waxy* stocks and the intercrosses. Pollen smears are prepared by breaking up a definite number of anthers (42) in a standardized iodine solution with a Virtis homogenizer. The pollen suspension is strained onto a large slide and covered with a 50 × 75 MM cover slip. Such a preparation usually contains about 50,000 pollen grains.

The smears are scored for the number of black-staining pollen grains (presumably *Wx*) present. These contrast markedly with the reddish brown color typical of *wx* grains. In the parental stocks the incidence of black grains is always low ($.42 \times 10^{-5}$ to 1.37×10^{-5} being the range). The analysis of the intercrosses is not yet complete, but apparently the incidence of black-staining grains depends upon the cross. Some crosses show no increased number of black grains over the parent stocks while others show a significant increase. The increased numbers of dark-staining grains in some crosses suggest the complexity of the *Wx/wx* locus.

Neufach, A. A. *See* Prokofieva-Belgovskaya, Alexandra.

Newcombe, H. B., A. P. James, and S. J. Axford. Genetic Hazards and Vital Statistics. *Atomic Energy of Canada Limited, Chalk River, Ont., Canada.*

The genetic damage to a human population resulting from a given exposure to radiation could be estimated with much greater confidence if we knew what part of our present hereditary ills and handicaps are due to recurrent natural mutations, and what part to differentials of fertility in the form of higher reproductive rates in the seemingly unaffected carriers of the genes. A method has been proposed by the Biology Branch of Atomic Energy of Canada, Limited, for surveying large human populations in order to distinguish between the respective roles of selection and mutation in maintaining the present load of hereditary defects.

The method uses the very large volume of routinely collected records of marriage, birth, ill-health, and death, to derive individual family fertilities and correlate these with the occurrence of various diseases. Mechanical or electronic handling procedures and a system of "family name codes" (or "genetic name codes") are used to bring these records (e.g., in punch card or magnetic tape form) into family groups.

A plan for a test study in the Canadian Province of British Columbia (present population about 1,400,000), based on marriage records over a ten-year period (1946–55), illustrates the technical and practical aspects of the undertaking. The children from these marriages would number about 150,000 by the end of 1958. Of these 10,000 would be stillborn, die in infancy, or appear in the register of crippled and handicapped children by the end of 1959; and a further 10,000 would be born with abnormally low birth weights, a characteristic known to be correlated with early death. About 30 to 40% of the stillbirths, infant deaths, and early defects, considered collectively, are believed to be hereditary; likewise, causes of birth weight variations are believed to be about 40% genetic. Thus, appropriate population studies would permit very substantial numbers of families to be used in the detection of fertility differentials.

Nicoletti, Benedetto, and Angela Solima. The Role of Selective Mating in Artificial Populations of *Drosophila melanogaster. Istituto di Genetica dell'Università di Napoli, Naples, Italy.*

The results of many experiments (made with the help of G. Morpurgo) led us to the belief that selective mating cannot be considered as the only and main factor for the gene-frequencies evolution in artificial populations of *Drosophila melanogaster.* C. Petit (1954) came to the same conclusion, emphasizing that during the competition among Bar and normal individuals, the elimination of mutants was due not only to a coefficient of sexual selection but also to a high coefficient of larval selection. The results of recent experiments performed with isogenic strains (normal and Bar 39th brother-sister mating) show that, besides these two coefficients of selection, other components of fitness must be considered.

Measurements of fecundity, fertility, longevity, hatchability, female and male

sterility show that Bar homozygous are in respect to the normal inferior in these characters too.

As a reasonable conclusion we think that a sexual coefficient of selection alone cannot account for the evolution of laboratory populations. The components are many and certainly not negligible and these, together with the possibility of sexual selection, contribute to the determination of relative fitness of individuals of a given genotype.

NILAN, R. A. *See* BLICKENSTAFF, JOYCE; KAMRA, OM P.

NODA, SHOZO. *See* HAGA, TUTOMU.

NORMAN, A. *See* SIEGEL, ALBERT.

NORONHA-WAGNER, M. *See* CÂMARA, A.

NUFFER, M. G. A Crossover Analysis of Mutable Alleles at the *A* Locus in Maize. *University of Missouri, Department of Field Crops, Columbia, Missouri, U.S.A.*

In order to determine the nature of the factors involved in mutable behavior at the A_1 locus a heterozygote composed of two distinguishable mutable alleles, one *Dt*-influenced and one *Ac*-influenced, but lacking these mutator factors, was prepared. One segment (a *a sh*) carried a (the dilute component of the compound A^b *allele*), *a* (a *Dt*-responding component of the A_1 locus) and sh_2 (a recessive shrunken endosperm character) all within a map distance of 0.25 crossover units. The other segment ($a^m Sh$) carried either a^m*-1* (a *Dt*-responding highly mutable component of *A*), a^{m-3} (an *Ac*-responding allele), or a^{m-4} (another *Ac*-responding allele), depending on the experiment, and Sh_2. The heterozygote was crossed by the complete recessive a^s *sh*. Progeny from the a^m*-1* heterozygote included the crossover types a a^m *Sh*, *a a Sh*, a*-Sh*, a^m *sh*, *a sh*, and a^m *a sh* which showed that pairing was random at the *A* locus in this case. The phenotype of the a^m *a sh* crossover cases indicated that the two mutable alleles can mutate independently even when closely associated on the same chromosome segment. Progeny from the a^{m-3} and a^{m-4} heterozygotes gave similar crossover types except that the a a^m *Sh* class was missing (the reciprocal *a sh* class was indistinguishable), suggesting preferential pairing. The frequency of crossing-over was strikingly different in the a^{m-3} heterozygotes. The rates expressed in crossover units were a^m*-1*, .11; a^{m-3}, .013; and a^{m-4}, .079. This may be due to the type of event involved in the origin of a^{m-3}. Preliminary investigations reveal no change in the mutability or phenotype of these alleles that can be attributed to crossing over. (Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 1860. Approved by Director.)

OAKBERG, EUGENE F. The Effect of X-Rays on the Mouse Ovary. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

Past work has shown that female mice have an average of 4 litters after an acute exposure to 50 r, then become permanently sterile; whereas in males, fertility is regained after doses of 1,000 r or more. Adult female hybrid (101 × C3H) mice killed 3 hours to 70 days after 50 r of X-rays were used to determine the basis for the different response of the ovary.

Early oocyte stages are extremely sensitive, but as the follicle develops the contained oocyte becomes more resistant to radiation-induced necrosis. All the earliest stages had degenerated and completely disappeared in 48 hours. Extensive degeneration of oocytes also occurred in follicles with a single layer of cells, but rare survivors were observed. Oocytes in follicles with several layers of cells showed an increased degeneration after 24 hours, but the number of normal cells was still 60% of control at 20 days. No increased degeneration of oocytes was observed in follicles with an antrum, but the number of mature follicles gradually declined as a result of failure of replacement from younger stages. No new formation of oocytes was observed, supporting the theory that oogonia do not persist in the adult. Thus the extensive regeneration characteristic of the irradiated testis cannot occur. Also, the genetic effects of irradiation of adult female mice cannot involve the oogonial stage.

Since the first meiotic division is not completed until shortly before ovulation, almost all germ cells in the adult ovary are primary oocytes. These oocytes are remarkably uniform in appearance, all being in the dictyate stage. Thus, apart from irradiations made shortly before fertilization, genetic effects will involve only this stage. This should be borne in mind when comparisons are made with male mice or with females of species where a different spectrum of stages is present.

OHKURA, KOJI. *See* TANAKA, KATUMI.

OKAMOTO, M. *See* SEARS, E. R.

OKSALA, TARVO A. Segregation and Missegregation of Chromosomes in the Female Meiosis of *Drosophila melanogaster*. *Institute of Genetics of the University of Helsinki, Helsinki, Finland.*

The author's investigations on the mechanism of non-disjunction have shown that it is highly probable that proximal heterochromatic blocks have a tendency to pair indiscriminately in the meiosis of the female *Drosophila melanogaster*. As a consequence, the centromere regions of even heterologous chromosomes often come into contact with each other. Sometimes this results in a missegregation (non-disjunction) of the chromosomes, which in turn leads to the formation of non-viable zygotes. The frequency of eggs of this type is different in inversion heterozygotes from that in structural homozygotes. The differences are explicable on the assumption that a bouquet orientation prevails in the early meiotic stages.

Olah, L. v. Cytological Investigations in *Corypha* Palms. *Biology Department, Duquesne University, Pittsburgh, Penn., U.S.A.*

The meiotic division of two monocarpic palms, *Corypha umbraculifera* L. and *C. elata* Roxb., was investigated in the Botanic Garden of Bogor (Buitenzorg), Java. Two phenomena were observed in both species investigated: (1) the formation of nucleolar buds on the nucleolar organizing region, during prophase of the first division of PMCs; (2) the appearance at zygotene of a Feulgen-positive substance, on the same region, or on the upper segment of the SAT-chromosomal threads attached directly to the nucleolar bulk.

A statistical approach was made to follow the formation and the disappearance of the nucleolar buds. Based on the observation of 9312 PMCs of *C. elata*, it was found that the budding process starts at leptotene and culminates at pachytene. The majority of the buds disappear in *C. umbraculifera* before diakinesis whereas in *C. elata* they disappear somewhat later.

The attachment of the SAT-chromosomes is collar-like on budded nucleoli, being located at the narrowest part of the budding nucleolus, i.e., between the nucleolus and the bud. In *C. umbraculifera* a darkly stained Feulgen-positive substance appears at this region, and becomes very conspicuous at early zygotene. Later on it seems to leave this place, often taking the shape of dot-drop or of rod-like bodies. These semi-liquid bodies seem to flow along the threads and subsequently are found among them. But in *C. elata* this substance appears at the upper segment of the SAT-threads, attached directly to the nucleolar bulk, and its quantity is noticeably less; consequently the above-mentioned motion is hardly observable. In both species this Feulgen-positive substance disappears at the end of zygotene.

The metaphase and the subsequent phases are as usual. The haploid chromosome number of both species is 18. Since no secondary pairing was observed in either species they are not considered to be polyploid. This observation supports the view of Sato who suggests that the basic number in "Corypheae" is 18.

Of several hypotheses put forward to explain the origin of nucleolar buds, the above observations lend most support to the hypothesis of Resende.

Based on the above finding in *Corypha* palms regarding the formation of Feulgen-positive bodies, a new light is thrown on the question of the origin of the heterochromatic bodies.

Olive, Lindsay S., and A. J. H. Carr. Crossing Self-Sterile Mutants of *Sordaria fimicola*. *Department of Botany, Columbia University, New York, N.Y., U.S.A.*

Spontaneous and irradiation-induced mutants have been obtained which range from reduced fertility to complete sterility. Blockage of fertility may occur at the following stages: (1) ascogonial development, (2) perithecial maturation, (3) ascus maturation (with the asci or ascospores aborting), (4) ascospore germination (germ tubes aborting). With the aid of ascospore color mutants for markers, fertile crosses may readily be obtained by pairing certain self-sterile cultures with self-fertile cultures, in which case crossed perithecia are often produced in abundance, along with pure wild-type perithecia. Perithecia of the sterile genotype may or may not develop, depending upon the sterile mutant used.

Paired self-sterile mutant cultures, often of less than normal growth rate, produce fully fertile heterokaryotic mycelia that are wild-type in appearance. These mycelia, in the crosses studied so far, produce crossed perithecia and perithecia of one of the sterile parental genotypes, the number of crossed perithecia often exceeding two-thirds of the total (preferential cross-karyogamy), or else they produce only crossed perithecia. An analysis of the first type of cross has shown that sterility is controlled primarily by two non-allelic, unlinked loci, one in each parental strain. In the latter cross, one parental strain carried a single locus for sterility, while the other carries two, both of which cause ascospore germ-tube abortion. Since these crosses involve non-allelic mutant loci and yield some self-fertile recombinants, they represent an *unbalanced heterothallism*, somewhat similar to that known to occur in a number of fungi in nature.

It is suggested that *balanced heterothallism* may have arisen repeatedly in nature by crossing over within a sterility locus in a cross of sterile by wild-type homothallic, or by the pairing of hetero-allelic sterile mutants.

ONO, HUMIHIKO. Chromosome Breakage induced by Hybridization. *Department of Biology, Faculty of Science, Tokyo Metropolitan University, Tokyo, Japan.*

The intergeneric hybrid, *Paraixeris denticulata* ($n = 5$) $\times$ *Lactuca squarrosa* ($n = 9$) considering its parental chromosome numbers would be expected to have 14 choromosomes. But its karyotype fluctuates between 10 and 14 chromosomes (Ono, 1943). The cause of this phenomenon is considered to be fragmentation and elimination of paternal chromosomes in the maternal cytoplasm. Such a phenomenon is also observed in other hybrids, such as *Paraixeris denticulata* $\times$ *Crepidiastrum keiskeanum* ($n = 5$), and *Crepidiastrum platyphyllum* ($n = 5$) $\times$ *Lactuca squarrosa*. The eliminated chromosomes seem to be the paternal ones. Recent studies of natural hybrid swarms of *Paraixeris* and allied species have revealed that such fragmentation and elimination of the chromosomes takes place in nature as well (Ono, 1957).

There are some regularities in the mode of fragmentation. In the hybrid, *Paraixeris denticulata* $\times$ *Lactuca squarrosa*, among various karyotypes, cells with a definite karyotype with 12 chromosomes are most abundant. Among these 12 chromosomes 2 are very small ones, probably proximal parts of some larger chromosomes. B-chromosomes have appeared as the result of hybridization. The loss of these small chromosomes not being fatal for the individual, some have a balanced karyotype of 10 chromosomes.

To ascertain which chromosome has actually fragmented and at which point the breakage has occurred, analysis of minor karyotypes has been carried out. By placing the plants in a room at 0°C for 3 to 4 days, starvation of nucleic acid in chromosomes was induced. Such chromosomes reveal characteristic achromatic bands by which individual chromosomes can easily be identified. This analysis indicates that a chromosome with characteristic patterns tends to break more frequently than the others and further that a plant in nature with the seemingly standard chromosome number of 10 can be shown to be a mixture of groups with different patterns. It is even possible to trace the ancestry of the hybrid by means of such patterns.

OSHIMA, CHOZO. The Resistance of Strains of *Drosophila melanogaster* to DDT and Dieldrin. *National Institute of Genetics, Misima, Japan.*

The strains of *Drosophila melanogaster* used in this experiment were started by different females captured in a single collection from each of several natural populations in Europe, Africa, Japan and United States. These strains have been cultured by mass-mating for about four years at Cold Spring Harbor and in Japan.

Forty female flies four or five days old, grown under optimal conditions, were exposed to DDT, Dieldrin or control test paper in a small vial. These test papers were prepared by the World Health Organization in Geneva. After exposure, flies were transferred into a fresh vial containing a wet paper and the number of dead flies was observed at definite intervals. Flies began to die by desiccation and starvation 25 hours after exposure to control paper.

The levels of DDT resistance in 18 strains of 10 localities were decided on the basis of percentages of accumulated mortalities 25 hours after exposure to 4% DDT test paper for 5, 10 or 15 hours. On the other hand, the levels of Dieldrin resistance in 11 strains of 5 localities were determined by percentages of accumulated mortalities 25 hours after exposure to 0.4 or 0.8% DL test paper for 0.5, 1.0, 2.0 or 3.5 hours. The several points showing each total average mortality of these strains at several time doses are nearly linear on normal logarithmic section paper. Resistance to both insecticides appears to be normally distributed in populations.

Eighteen strains were classified into three groups, non-resistant, slightly resistant, and resistant to DDT, and the numbers of dead flies at 25 hours after exposure to 4% DDT test paper for 15 hours were analysed statistically as to locality and strain. Highly significant differences in DDT-resistance between localities and between strains were found; the latter was larger than the former but not significantly so. Eleven strains were also classified into three groups, non-resistant, slightly resistant, and resistant to Dieldrin and the numbers of dead flies at 25 hours after exposure to 0.8% DL test paper for half hour were analysed statistically as to locality and strain. Highly significant differences in DL-resistance between localities and between strains were found once more, but in this case the former was significantly larger than the latter.

These results indicate conclusively that any local population is extremely heterogeneous in DDT and Dieldrin resistances; the locality-strain differences in the two cases may be attributed to different uses of the insecticides.

OSTER, IRWIN ISAAC. Frequency-Dosage Relations for Mutations following X-Irradiation of Sensitive and Resistant Germ Cells. *Indiana University, Bloomington, Ind., U.S.A.*

Although many experiments have served to confirm Oliver's (1930) initial demonstration of the linear relation between the frequency of recessive lethal mutations induced by X-rays in sperm of *Drosophila* and the dosage of radiation used, it can be inferred from Auerbach's and Lüning's recent work that most, if not all, of the previous studies dealing with this problem involved the treatment of heterogeneous samples of germ cells. Since it is now well established that even the post-meiotic stages of spermatogenesis of *Drosophila* differ tremendously in their radiosensitivity and since knowledge of the relationship of lethal mutation frequency to

dosage employed has a bearing on the interpretation of mutational mechanisms it was decided to reinvestigate this problem with the latest genetic techniques available. Homogeneous samples of the most radiosensitive and the most radioresistant stages of gametogenesis, represented by spermatids and oögonia, respectively, were treated. X-raying spermatids yielded 195/8,978 (i.e., lethals among tested chromosomes) for 250 r and 130/1,859 for 1,000 r, while the controls gave 19/2,392. This gives induced rates of 1.38 ± 0.24% and 6.20 ± 0.39%, indicating a linear relationship. The translocation frequencies obtained were 1.32 ± 0.20% (45/3,421) and 9.65 ± 0.75% (148/1,533), thereby showing a rise for these multi-break events proportional to $(\text{dose})^{1.4}$. Irradiation of oögonia in third instar larvae yielded 117/16,064 for 600 r and 33/2,056 for 2,400 r, while the controls gave 49/11,630. This gives induced rates of 0.31 ± 0.09% and 1.19 ± 0.30%, again a linear relationship. For equal doses, spermatid frequencies were 12 times oögonial frequencies. Special methods ruled out the presence of preexisting lethals as well as the inclusion of large clusters of mutations arising from one mutated cell by mitotic division. Thus at the dosages studied the strictly linear relation of lethal frequency to dosage employed was upheld for the most radiosensitive germ cells and extended to the least sensitive ones known in *Drosophila*.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the U.S. Atomic Energy Commission, Contract AT(11–1)–195.)

Paik, Yong Kyun. Seasonal Changes in *Drosophila* Populations in Two Adjacent Areas in Korea. *Laboratory of Genetics, Department of Biology, National Chunnam University, Kwangju, Korea.*

Seasonal changes in population size of *Drosophila* in South Korea had been checked over a period of a year, from July 1956 to June 1957. To confirm any areal nonhomogeneities in the distribution or seasonal changes of the species, two woodland areas were selected which are isolated from each other by a mountain, and which are about 700 meters apart.

A total of 12,918 flies was collected during the period in both areas. Area I provided 6,082 flies representing 27 sympatric species of which 19 belonged to the genus *Drosophila* and 8 to the other genera. In area II 6,836 flies were collected representing 25 sympatric species of which 16 belonged to the genus *Drosophila* and 8 to the other genera.

Changes in the total populations showed two sharp seasonal maxima in its size, one in the autumn (October-November) and the other in the spring (April) in both areas. Total population sank to an extremely low level, statistically zero in its size, during the cold winter months (December-February), which can be generally considered to be a severe "population bottle-neck period" in our climate. Total population also dwindled to the low level during the warm summer months (July-August). Total population changes from month to month through the year are also closely correlated with each other in both areas ($R = 0.969$).

Specific changes are also considered for 8 species which were abundant or common in the relative frequencies in the two populations. In both areas, *D. auraria*, *D. transversa* complex, *D. nigromaculata* and *D. cheda* complex showed two yearly maxima in autumn and spring: *D. bizonata* in winter and spring: *D. coracina*, *D. lutea* and *D. suzukii* peaks in spring, autumn and autumn respectively.

Specific fluctuations from month to month in both areas showed statistically a positive correlation between the same species.

D. bizonata is a representative species in these areas and has been continually collected throughout the year, even in months of severe cold (December-February) during which none of the other seven selected species was collected.

Deviations from a sex ratio of 50:50 have been examined for the 8 selected species. Comparing the results obtained in both areas, it may be noted that the deviation, in general, is statistically striking but is apparently species specific.

PALIWAL, R. L., and BEAL B. HYDE. The Association of a Single B-Chromosome with Male Sterility in *Plantago*. *Department of Plant Sciences, University of Oklahoma, Norman, Okla., U.S.A.*

Indian strains of *Plantago ovata* and *P. coronopus* contain a low percentage of completely male sterile plants. In male sterile *P. ovata* ($2n = 8$) meiosis proceeds normally, but the microspores later degenerate. To determine the nature of the sterility such male sterile plants were crossed with male fertile plants. Several generations of controlled crossing indicate that the sterility is cytoplasmic.

In *P. coronopus* our chromosome counts showed two karyotypes: $2n = 10$ and $2n = 10 + 1$. The odd chromosome in the $2n = 10 + 1$ type is shorter than, and does not pair with any other chromosome of the normal complement. Since it is almost entirely heterochromatic it may be considered a B-chromosome. The two strains are identical except that those containing B-chromosome are completely male sterile. The anthers never protrude from the flowers and seed set on bagged plants is negligible. At the first division of meiosis the B-chromosome does not lag behind and is incorporated into one of the daughter nuclei. The second division is normal, but the microspores degenerate.

The $2n = 10 + 1$ plants are apparently facultatively apomictic. Attempted crosses between male sterile *P. coronopus* and *P. amplexicaulis* ($2n = 10$) produced good seed set. All the plants in the "hybrid" progeny, however, resembled the female parent in all respects. Out of a total of 30 plants only one was male fertile and $2n = 10$, while the rest were male sterile and $2n = 10 + 1$. The single exception may have resulted from contamination of *P. amplexicaulis* pollen with that of *P. coronopus*.

This is the first case we are aware of in which male sterility may be attributed to the presence of a single B-chromosome.

PALIWAR, R. L. *See* HYDE, BEAL B.

PANDEY, KAMLA KANT. Microsporogenesis, Time of *S* Allele Action, Pollen Cytology and Place of Inhibition and Their Relationship with the Evolution of Gametophytic and Sporophytic Systems of Self-Incompatibility. *Department of Botany, Ohio State University, Columbus, Ohio, U.S.A.*

Self-incompatible plants have been variously subdivided into two groups according to some prominent characteristic. One that is based on the nature of the *S* gene

action is composed of (1) gametophytic system—in which the reaction of the pollen is determined by the pollen genotype, and (2) sporophytic system—in which the reaction of the pollen is determined by the genotype of the mother plant. The basic difference between the two systems lies in the time of the *S* allele action. In the sporophytic system the *S* allele action takes place at least before anaphase I and in the gametophytic system at or following anaphase I. Pandey has suggested that the time of expression of *S* allele action in the sporophytic system is after anaphase II and may be either before the separation of the four microspores or after cytokinesis but before the development of exine. In the gametophytic system the time of expression is either after cytokinesis or after exine development. Since in the sporophytic species the microspore wall is laid down from the cytoplasm in which the *S* alleles have already expressed, the inhibition occurs at the contact of the pollen grain with the stigma which has correspondingly specific substances. In the gametophytic species the *S* allele action occurs internally in the individual microspores and thus inhibition in these species occurs after pollen germination when the specific substances of the pollen tube and the style are brought together.

The above hypothesis amounts to precocious nuclear activity in the sporophytic system. It is supported by the finding of Brewbaker who discovered that the gametophytic species had binucleate pollen grains, whereas the sporophytic species had trinucleate grains. However, there are several exceptions. The gramineaecious species are trinucleate, but three such species have the gametophytic system. All the self-incompatible heterostylous species have sporophytic system, but some of these species have binucleate grains. This hypotheses will be further discussed.

PARSONS, P. A. On the Phenomenon of Genetic Interference. *Department of Genetics, University of Cambridge, Cambridge, England.*

The phenomenon of genetic interference has been a topic of discussion and speculation ever since its discovery by Muller in 1916. In recent years a rational theory of genetic interference has been developed by Owen which was shown by him to fit existing *Drosophila melanogaster* data. Maize data examined in a similar manner agree with Owen's model, but the data are of insufficient accuracy to detect any minor deviations from it.

However, some recent linkage tests in the house mouse of more critical nature for linkage groups V and XIII show that modifications of the theory may be necessary. In both of these linkage groups there is a sex difference, the males exhibiting more intense interference than the females. This effect is most marked in linkage group XIII. Furthermore, on Owen's model, a recombination fraction greater than 50% would be expected between the extreme markers of linkage group XIII in the females, but this was not found. Such long chromosome arms are of extreme value in testing the validity of any metric.

It therefore appears that a different metric is needed for each sex, and possibly for each chromosome of the house mouse. Furthermore, variation in environment will act by changing the metric to a certain extent. Changes in the metric cause larger variations in recombination in the centromeric regions than elsewhere. This is supported by the effects of temperature and age on recombination in *Drosophila* both of which produce their effects in the centromeric regions.

PARTANEN, CARL R. The Quantification of a Tumorous Response to Ionizing Radiations in Fern Prothalli. *Children's Cancer Research Foundation, Boston, Mass., U.S.A.*

Tumors occur normally at a very low rate in the *in vitro* culture of certain fern prothalli, notably *Pteridium aquilinum*. This rate can be greatly increased by applying an optimal dose of ionizing radiations, either to actively growing prothalli or to dormant spores. This system presents a unique opportunity for the analysis of factors involved in the process of tumorization since the response occurs in a haploid organism *in vitro* on completely defined media in a readily controlled physical environment. The primary causal event is known to occur in a single cell and appears to be a mutation. The radiation effect persists in dormant spores over long periods of storage prior to germination. Further, this process lends itself readily to quantitative analysis since spores can be irradiated in quantity and then sown in known amounts. Work to date has consisted primarily of developing rather precise quantitative techniques in which the exact size of the inoculum is known and also the absolute number of viables can be determined to a high degree of accuracy. Present studies are directed towards the determination of the dosage-response relationships and also towards the screening for substances which may in any way alter the tumor response to radiations. Preliminary results indicate that a linear relationship exists between the amount of radiation and the resultant number of tumors, for doses up to 16,000 r.

PAVLOVSKY, O. *See* BEARDMORE, J. A.

PELC, S. R. *See* LA COUR, L. F.

PENHA, A. M. *See* SCHREIBER, GIORGIO, *et al.*

PEREGRINE, W. T. H. *See* GRIFFITHS, D. J.

PERKINS, DAVID D. Crossing-over in Linkage Group I of *Neurospora. Department of Biological Sciences, Stanford University, Stanford, Calif., U.S.A.*

The discovery of new loci has extended the arms of linkage group I of *Neurospora crassa* in both directions to a total length exceeding 125 units, within which about thirty useful markers have been mapped. The following information has been obtained by analysing random segregants and tetrads from multiple point crosses. Chiasma interference is positive for neighboring short regions, at least within the range of interval lengths examined, where recombination frequencies are such that non-selective analysis is practical. The intensity of interference is similar in the right arm of group I in *Neurospora* and in the X-chromosome of *Drosophila*

melanogaster, which is of about the same genetic length. Post-reduction of alleles at loci increasingly far from the centromere (as determined from tetratype frequencies with respect to a centromere marker) increases to a maximum value greater than two-thirds, and then declines so as to approach this random limiting value at distances beyond the maximum. This is the pattern that would be expected to result from positive chiasma interference. Crossover frequencies are highly variable for similar intervals in crosses of different parentage. Because of these variations of intrinsic origin, conclusions regarding gene order may be unreliable if based on 2-point rather than on 3-point tests. (This work has been supported by grant E1462 from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.)

PERLIN, SEYMOUR, and GORDON ALLEN. Designed Genetic Bias in a Schizophrenic Sample. *National Institute of Mental Health, Bethesda, Maryland, U.S.A.*

For a disease that is etiologically diverse, the best clues to pathogenesis may come from biased samples. A sample may be usefully biased by restriction to part of the diseased population, but for some purposes a bias in the opposite direction is better, bringing in all sub-types of the disease regardless of their relative frequency. This is true in the screening of new tests in the hope that one will detect a gross biochemical defect related to some form of the disease under study.

If schizophrenia is etiologically diverse, cases with a simple genetic cause are the ones most likely to have underlying gross chemical pathology. In planning the selection of 16 schizophrenic patients for intensive biochemical investigations, we decided that the widest sampling of genetic causes would be achieved if one-fourth showed a family history suggesting dominance; one-fourth, a history suggesting recessiveness, and one-half, a negative family history. We reasoned that any common genetic type would be certain to occur among the patients with affected close relatives, but that any possible semi-lethal dominant or rare recessive type might be crowded out of these groups if much schizophrenia is due to a common dominant defect, to polygenes, or to social causes. The patients with negative family history would then be more likely to include an uncommon genetic type, and they could, by design, also include any distinct clinical types not found in the first half of the sample.

PERRY, T. O. *See*, WANG, CHI-WU.

PERSON, CLAYTON. A Study of Asynapsis and Its Effect on Subsequent Chromosome Behavior. *Canada Department of Agriculture Research Laboratory*, and *University of Manitoba, Winnipeg, Man., Canada.*

From earliest prophase onward the chromosomes in meiotic cells of a barley plant (*Hordeum vulgare* L., $2n = 14$) remained unpaired. At the stage corresponding to metaphase of first meiosis quasibivalents were not found and, for those cells in which poleward movements of the univalents had taken place, the distribution

of univalents was random. These observations support the author's earlier conclusions, first, on the processes leading to the formation of quasibivalents and second, on the effect of quasibivalents, when present, on the polar distribution of univalents.

PETERS, H. F. Hybridization of Domestic Beef Cattle and American Bison. *Canada Range Experimental Farm, Manyberries, Alberta, Canada.*

In 1915 the Canada Department of Agriculture began a project to develop a range beef animal for western and northwestern Canada that would combine some of the hardy characteristics of the American bison and the meat qualities of domestic beef cattle. The experiment was conducted at Wainwright, Alberta, until 1950, when the herd was moved to the Range Experimental Farm, Manyberries, Alberta. The project is being continued at Manyberries with a herd of 130 cows of ½-bison to $\frac{1}{16}$-bison breeding.

Male sterility has prevented the development of a breed of Cattalo. No fertile first-cross bulls were obtained. Semen from ⅛-bison to ¼-bison bulls varies in quality from complete absence of spermatozoa to normal concentration and a small proportion of the bulls show varying degrees of fertility. The cause of sterility has not been determined.

A study of foraging performance of beef cows on winter range in 1951–2 showed that the Cattalo foraged on upland open range more frequently than the cows of domestic breeds. Also, a greater proportion of Cattalo cows were out foraging as compared with cows of domestic breeds. The amount of foraging on upland range (as opposed to sheltered coulees) increased significantly with increase in temperature, and decrease in wind mileage, and amount of supplemental feed. Temperature was the most important of environmental factors, wind mileage and amount of supplemental feeding having a significant effect only at low temperatures.

In feedlot studies during 1952–3 and 1956–7, Cattalo calves made significantly greater feedlot gains than bison and lower gains than Herefords. Progressively lower carcass grades, lower proportions of carcass weight in the hindquarters, and an increase in dressing percentage were obtained as the proportion of bison breeding increased.

PETERSEN, J. A. *See* CORDEIRO, A. R.

PFAHLER, PAUL L. and JULES JANICK. A Proposed Method for the Determination of Dominance Levels. *Purdue University, Department of Horticulture, West Lafayette, Indiana, U.S.A.*

A method to determine the level of dominance by the use of euploidy and aneuploidy is proposed. If tetraploids can be produced artificially from homozygous diploid inbreds AA and BB, the triploids, AAA, BBB, AAB and ABB, could be synthesized from appropriate crosses. The mean of the triploid hybrid AAB can be partitioned into three main effects: (1) the mean of the diploid hybrid, AB; (2) the effect of triploidy, 2(AAA-AA) + (BBB-BB)/3; (3) any effect de-

signated I^{gA}, brought about by the additional A genome. The I^{gA} value can be estimated by the following equation, I^{gA} = AAB-AB-[2(AAA-AA) + (BBB-BB)/3]. The I^{gB} value for the triploid hybrid ABB can be estimated similarly. The level of dominance can be estimated by a comparison of the sign and magnitude of the I^{gA} and I^{gB} values. If the mean of the diploid hybrid AB were within the range of both parental means indicating additive or partially dominant gene action, the sign of I^{gA} and the I^{gB} values would be positive. The difference between the I^{g} values would indicate a difference in the contribution of dominant alleles from each parent. If the mean of the diploid hybrid AB were as large or larger than either parent, dominant or overdominant gene action would be operative. If gene action were dominant, the I^{gA} and the I^{gB} values would be zero. If gene action were overdominant owing to divergent physiological function of alleles, the I^{gA} and the I^{gB} values would be positive. If the gene action were overdominant owing to optimum level of gene product, the I^{gA} and I^{gB} values would be negative. If the production of trisomics were possible, a similar analysis could be employed to partition the I^{g} effect into individual chromosomal components. The results obtained by this method would not be influenced by epistasis or linkage.

PHILIP, URSULA. Genetics and Cytology of the Seaweed Fly, *Coelopa frigida* (F). *Department of Zoology, King's College, University of Durham, Newcastle upon Tyne, England.*

Coelopa frigida (F) is a fly living in wrack beds, which are periodically thrown up by the sea. This specialised ecological niche gives the unique opportunity to investigate the genetic structure of a species, the distribution of which is almost strictly linear. During the last years my collaborators and I have been studying the biology, cytology, and genetics of this fly.

Coelopa frigida has a life cycle of 12 days at 25°. The eggs are laid in batches of approximately 80. There is, as a rule, a high percentage of hatch. The animals show a great deal of variability in response to environmental conditions. The three varieties described in the systematic literature are produced according to the amount of food available during larval development. The sex ratio is of great interest. The primary sex ratio, estimated from batches of flies, where mortality between egg and imago was very low, is approximately .5 with a variance much below what would be expected by chance. With rising death rate due to over-population the variance increases. In cultures with gross over-population there is a significant deficiency of males.

We have five stocks with visible mutants; "white eye," "sherry eye," "cut wing," and "chopped bristles" were collected from free living populations, the dominant "Delta" from laboratory cultures. No sex-linked mutants have been found. Three egg lethals have repeatedly been isolated from one free living population. One lethal causes death at an early stage, one at the time of the involution of the head, and a third one at the time of eclosion. The Delta homozygote dies after faulty cleavage at the end of gastrulation.

Investigations of the salivary gland chromosomes have shown three populations to be heterozygous for multiple inversions.

PIPKIN, SARAH BEDICHEK. A Study of Male-Determining Regions of Chromosome 3, *Drosophila melanogaster*. *Howard University, Washington, D.C., U.S.A.*

A search was made for male-determining regions of chromosome 3 in *Drosophila melanogaster*. Males heterozygous for either one or two 3,4 translocations were crossed with homozygous y^2; ru ca triploids. Recessive markers in the male parents allowed the detection of hyperintersexes (2X3A + fragment of 3), hypointersexes (2X3A — fragment of 3), and other aneuploid progeny. The distribution of Dobzhansky's six intersex sex types in hyperintersexes has been studied in nine short regions of chromosome 3. Experiments were carried out at 22° ± 0.5°C. Chi square homogeneity tests were made on the frequencies of sex types of control intersexes hatching in different bottles with the same male parent composition. Where such frequencies proved homogeneous (nearly always), they were pooled. Hence a comparison of these pooled sex type frequencies of control 2X3A and hyperintersexes of the same cross reveals a sex shift (or lack of it) due to the presence of the extra fragment rather than to that of the genetic modifiers. A significant shift towards maleness in hyperintersexes was found in 4 regions, 3 of which overlap: (1) region c–89E (in salivaries, between 86D and 89E), mean sex type of hyperintersexes, 1.3409 ± 0.072, of control 1.6993 ± 0.061; (2) region 2R (from end of 94A to end of chromosome), mean of hyperintersexes, 1.2449 ± 0.073, of control intersexes, 1.9933 ± 0.051; (3) region 30R (from end of 96F to end of chromosome), mean of hyperintersexes, 1.4350 ± 0.065, of control intersexes, 2.2560 ± 0.071; (4) region H5R (from point intermediate between 94A and 96F to end of chromosome), mean of hyperintersexes 1.1905 ± 0.084, mean of control, 1.9535 ± 0.090. No shift of intersex sex types in hyperintersexes as compared with their control 2X3A was found in the four regions analysed statistically: 89E-28 (between 89E and 92E); 85C-c (between 85C and 86D); 12–13 (between 67F and 73C); 28–2 (between 92E and end of 94A). No shifts towards femaleness existed in any of the hypointersexes found for regions 28-2, 85C-c, 12-13, c-89E, 85C-89E, and 89E-28. No intersexuality was found in the hypertriploid females (3X3A + fragment of 3) which survived for all regions.

PITTENGER, THAD H. Mitotic Instability of Pseudo-Wild Types in *Neurospora*. *Oak Ridge National Laboratories, Milwaukee, Wis., U.S.A.*

In *Neurospora* it is known that phenotypically wild-type progeny from crosses of closely linked mutants are not always recombinants, but some appear to originate from n + 1 nuclei. Such disomic nuclei are unstable and by some post-meiotic process give rise to genetically different haploid nuclei. These strains are then heterokaryotic and are referred to as pseudo-wild types (PWT). In an attempt to localize the time and to characterize the mechanism involved in the mitotic breakdown of disomic nuclei, an analysis was made of the nuclear ratios present in mycelia derived from two or more individual hyphae just emerged from opposite ends of PWT ascospores from a cross of *lys*-3 × *nic*-2, *al*-2. Although a wide range (0-100%) exists in the proportion of *lys*-3 nuclei that can be found in the hyphae emerging from a disomic ascospore, there is a high positive correlation between the proportion of *lys*-3 nuclei found in the hyphae from opposite ends of a spore and this is similar to the correlation between the nuclear proportions

in different hyphae from the same end of a spore. This high correlation suggests that these proportions are established in the spores prior to the emergence of the hyphae. Since reduction must precede any inequality in the proportion of the two nuclear types, it is concluded that reduction takes place during early nuclear division inside of the ascospore. That it is very often, but not always, the first nuclear division within the ascospore that is abnormal is shown by a comparison of the frequency of PWTs and recombinant wild types in irradiated and non-irradiated populations. In populations in which the number of viable nuclei per spore had been effectively reduced from two to one by X-irradiation, the proportion of phenotypically wild types which were PWTs was significantly reduced, suggesting that many binucleate PWT spores were already heterokaryotic.

PIZA, S. DE TOLEDO. A Note on the Chromosomes of an Elaterid Beetle. *Esc. Sup. de Agricultura "Luiz de Queiroz," Universidade de S. Paulo, Brazil.*

The following observations have been made in the course of a study of male meiosis in the elaterid beetle *Pyrophorus nyctophanus* (Germ.).

The spermagonia have fifteen chromosomes of different sizes, the longest ones being clearly curved. The smallest element seen at metaphase is, quite probably, the sex chromosome. Primary spermatocytes exhibit at metaphase 7 dumbbell-shaped tetrads and a relatively small sex chromosome. Some bivalents, consisting of two chromosomes joined by what used to be considered a terminal chiasma, show at diplotene-diakinesis in each chromosome a median constriction, and despite that never formed ring-tetrads. Moreover, tetrads, which by their size correspond to the largest, curved and probably metacentric spermatogonial chromosomes, sometimes appear clearly quadripartite at metaphase, the two chromatids at each side of the equator, in disagreement with their metacentric condition, showing tapering ends independently connected with the corresponding pole. The sex chromosome passes undivided to one pole in the first division, so that secondary spermatocytes with 7 and 8 (7A + X) chromosomes have been found.

The author concludes that, if we admit that the median constrictions seen in bivalents at diakinesis do not represent centromeres and that division of the chromosomes into their chromatids takes place prior to pairing, the absence of ring-tetrads, and other peculiarities of elaterid meiosis will be satisfactorily explained.

PLOUGH, HAROLD H. *See* ROBERTS, MARGARET R.

PODDUBNAYA-ARNOLDI, V. A. Study of the Fertilization Process in Certain Angiosperms on Living Material. *Main Botanical Garden of the Academy of Sciences, Moscow, U.S.S.R.*

After the papers of Strasburger (1900) and Shibata (1902) on the fertilization in *Monotropa hypopitys* and *M. uniflora* no data on the study of this process on living material were to be found in literature. To fill up this gap the author has

studied fertilization and the processes connected with it in *Cypripedium insigne*, *Calanthe Veitchii* and *Dendrobium nobile* on living material. Comparison of the formation of gametophytes and gametes, and of fertilization observed on living and fixed material shows them to be very similar. Along with this fact, vital study enabled the elucidation of a number of details which could not be studied at all or could not be seen clearly enough on fixed material.

The presence of chondriosomes and colourless plastids was observed at different developmental stages of the pollen and ovule, micro- and macrospores, male and female gametes, as well as zygotes. The author concludes therefore that plastids and chondriosomes are the sites of synthesis of different plastic, physiologically active substances and enzymes. In pollen tubes "stoppers" were found which prevent the distribution of nutritive and energizing substances throughout the length of the tube stimulating their concentration at the tip of the pollen tube.

When localization and dynamics of different plastic substances in pollen, pollen tubes, embryo sacs and gametes were studied, a high fat content was found, particularly at the time of pollination and fertilization. This phenomenon may be connected with the fact that fat is not only a trophical substance but a solvent of carotinoides as well. The carotinoides appear to play an important part in generative process.

As progress in the study of fertilization on living material is as yet insufficient, further improvement of vital methods and selection of the most suitable objects of study are needed, particularly with respect to the fusion of the gametes.

POND, VIRGINIA. *See* WILSON, G. B.

PONS, JOSÉ. Quantitative Genetics of Human Dermatoglyphics: Frequency Distributions and Sib Pair Correlations of Palmar Main Lines Transverseness. *Instituto "Bernardino de Sahagún" de Antropología y Etnología, Barcelona, Spain.*

The general course of the main lines on the palm shows a variable approach to the transverse direction, i.e., the lines exhibit a greater or lesser degree of obliquity. Racial differences are found in this trait, and there is no doubt that it is inherited. In this paper we consider the problem of the quantitative expression of the degree of obliquity of palmar main lines, analysing their distribution and the deviations from normality. At the same time we consider the heritability by calculating the intraclass correlation among sibs.

A sample consisting of 400 Spaniards (200 ♂ and 200 ♀) shows a small negative skewness which is not significant, and a greater platykurtosis for the considered trait. The intraclass correlation among sibs, using data from 113 sibships with 286 members, is near 0.5, the theoretical value when additive genes with independent effect are present.

POULSON, D. F. *See* MALOGOLOWKIN, CHANA; KANEHISA, T.

PRAKKEN, R., and J. H. VAN DER VEEN. Flower Variegation and Seed Quality in *Nicotiana tabacum*. *Laboratory of Genetics, Agricultural University, Wageningen, The Netherlands.*

In F_2 of the cross between two pink-flowered varieties of *Nicotiana tabacum* about $\frac{1}{16}$ of the plants showed white-(pale) pink variegated flowers, together with bad, shrivelled seed. On selfing this pink variegated type and crossing it to both parents, it proved to be double recessive, vp1vp1 vp2vp2 (vp = variegated pale), the parents being Vp1Vp1 vp2vp2 and vp1vp1 Vp2Vp2.

Carmine colour (Pk) is dominant over pink (pk). The cross of carmine unicoloured with pink variegated, F_1 carmine, gave F_2 segregation (1,228 plants) into 45 carmine unicoloured: 3 carmine variegated: 15 pink unicoloured: 1 pink variegated, demonstrating for the carmine parent the genotype PkPk Vp1Vp1 Vp2Vp2.

A recessive white type (wh) appeared to be whwh PkPk Vp1Vp1 Vp2Vp2, versus WhWh pkpk vp1vp1 vp2vp2 of pink variegated.

The carmine variegated plants show a very large range of variation in number and size of the coloured or white spots and in intensity of colour. Different types of variegation may occur in one plant often in separate sectors, varying from small within flower sectors to distinct within plant sectors. Sharp classification has hitherto not been possible.

By rough estimation a colour-value was given to each carmine variegated plant, from 1 (almost white) to 9 (almost carmine), 10 being reserved for carmine unicoloured. In one experiment a sample of 21 carmine variegated plants, with colour-values from 1 to 9, gave offspring with mean family values from $\pm$ 3 to 7, clearly showing positive parent-offspring correlation, possibly discontinuous. Moreover, plants with values 6, 7, 8 and 9 gave a few carmine unicoloured (value 10) offspring; these carmines always gave similar offspring as related variegated plants; root cuttings produce but variegated plants.

From clearly sectorial plants dark and pale sectors (pairs of values 10–6, 10–5, 10–4, 6–3, 6–3, 5–3, 5–2, 5–2) were selfed. The resulting offspring gave no indication of positive correlation between parent sector value and mean offspring value.

In the carmine variegated type the relation between colour-value and seed quality was studied carefully: a strong positive correlation was found in individual flowers, plant sectors and plants. Recessive white plants (whwh) of the vp1vp1 vp2vp2 genotype can be recognized by their shrivelled seeds.

PREVOSTI, ANTONIO. Inversion Heterozygosity and Selection for Wing Length in *Drosophila subobscura*. *Centro de Genética Animal y Humana, Universidad, Barcelona, Spain.*

To obtain information about the structure of the genotype controlling adaptive traits, e.g., wing size (Prevosti, 1954, 1955) and to get some insight into the meaning of chromosomal polymorphism in relation to this point, the following experiment was carried out. Two lines of artificial selection were made of one stock of *D. subobscura*: one line selected for long wings, and the other for short wings. In the starting populations (F_1 reared in the laboratory of 25 wild flies of a natural population) the mean number of chromosome inversions per individual was 3.65. After 9 generations of selection the line selected for long wings had 3.80 inversions per individual and a sample of a control line 3.52.

The differences between these values cannot be interpreted as an increase of inversion number due to selection, since they do not exceed the expected sampling error. On the other hand the change is clear in the short wings line in which chromosomal homozygosity is virtually reached. In 25 individuals observed only one inversion was found—in the chromosome X. Two other lines of selection are being studied and are showing the same trend.

A comparison is made of these results with the changes in the selected trait and other correlated responses (especially in fertility and viability) obtained in these and other lines (Prevosti, 1956, 1958). We infer that the inverted segments of the chromosomes act in the same way as supergenes, in which the factors controlling size, viability and fertility are coordinated and give compound dominance of long over short wings and, probably, heterosis in viability and fertility. In this way chromosomal polymorphism represents a mechanism evolved to stabilize the coordination of a genotype adapted, in which heterosis has an important role.

PRIADCENCU, AL., I. TARNASKI, A. MELACRINOS, D. MELBER et E. BOLDEA. Quelques formes nouvelles de plantes obtenues par des croisements eloignés entre céréales. *Bucharest, Romania.*

Les croisements éloignés entre différents genres et différentes espèces de plantes donnent, par ségrégation, de nouvelles formes d'hybrides stables possédant certains caractères et des qualités des deux parents dont ils proviennent.

En 1938, plusieurs variétés de blé d'automne ont été croisées avec deux variétés de blé durum. Par sélection, nous avons réussi à isoler des générations avancées un hybride stable, nommé *speltoïde*. Le nombre $2n$ du speltoïde est de 35 chromosomes. A la métaphase de la division I, on observe généralement 17 bivalents plus 1 univalent. Les irrégularités observées au cours de toute la méïose ne se chiffrent qu'à 2.4%. Les caractères caryologiques de cet hybride semblent en voie de se stabiliser.

Entre 1939 et 1944, nous avons croisé 40 variétés et populations de blé d'automne avec la variété *Petkus* de seigle d'automne. Les hybrides de la F_1 sont bien développés mais stériles. Par rétrocroisements avec le blé et le seigle nous avons finalement obtenu un hybride stable et fertile que nous avons désigné *Néo-Sécalotriticum*. Son nombre $2n$ est de 56 chromosomes et à la métaphase I il possède 28 bivalents. C'est donc un amphidiploïde.

De 1937 à 1942 nous avons aussi croisé des espèces d'*Aegilops*, de *Triticum* et de *Sécale*. Un hybride de F_1 de *T. vulgare erythrospermum* $\times$ *T. durum* var. *valenciae* croisé à *A. ovata* nous a finalement donné un hybride stable désigné *Néo-Aegilotriticum*. Son nombre $2n$ est seulement de 42 chromosomes et il peut être considéré comme un triploïde.

Ces plantes n'ont pas d'importance économique. Elles sont cependant très utiles en amélioration des plantes et sont aussi d'un très grand intérêt scientifique.

PRICE, SAM. Chromosome Numbers Transmitted by *Saccharum officinarum*. *Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, U.S.A.*

"Triploid" individuals have rarely, if ever, been found in *Saccharum officinarum*

L., yet hybrids from *S. officinarum* L. × *S. spontaneum* L. have almost always had chromosome numbers that are the sum of their maternal diploid and paternal haploid numbers ($2n + n$). However, these observations have come largely from attractive individuals, selected and maintained in sugar-cane breeders' collections.

For unbiased information unselected progeny were studied. Of 94 individuals from intraspecific crosses with *S. officinarum* ($n = 40$), 93 had 80 chromosomes ($n + n$) and one (with $2n = 79$) was an aneuploid of the $n + n$ type. Of 100 hybrids from *S. officinarum* ($n = 40$) × *S. spontaneum* ($n = 32$), all had 112 chromosomes ($2n + n$). Of 27 *S. officinarum* ($n = 40$) × *S. spontaneum* ($n = 40$) hybrids, 26 had 120 chromosomes ($2n + n$), but one had 80 chromosomes, the $n + n$ number. Of 32 hybrids from *S. officinarum* ($n = 40$ × *S. spontaneum* ($n = 56$), 31 had 136 chromosomes ($2n + n$) and one (with $2n = 130$) was an aneuploid of the $2n + n$ type. Of 24 *S. officinarum* ($n = 40$) × *S. spontaneum* ($n = 64$) hybrids all were found to have 144 chromosomes ($2n + n$).

Observations upon unselected progeny agree with earlier results. In intraspecific crosses, *S. officinarum* rarely, if ever, transmits its diploid number maternally. Conversely, the same species rarely transmits its expected gametic chromosome number maternally when crossed with *S. spontaneum*.

If both n and $2n$ eggs are present in *S. officinarum* at the time of fertilization, selective fertilization and/or differential survival must be almost completely effective in eliminating "triploids" on the one hand and $n + n$ hybrids on the other. An alternative hypothesis might be the failure of egg and sperm nuclei to enter synchronously into the first division of hybrid zygotes.

PRITCHARD, R. H. Recombination and Negative Interference in *Aspergillus nidulans*. *Department of Genetics, University of Glasgow, Glasgow, Scotland.*

The use of selective techniques in a number of organisms has permitted an analysis of the properties of recombination in extremely short genetic intervals. In *Aspergillus nidulans* it is clear that, even between alleles, recombination is normally a reciprocal process. There is no evidence that intragenic recombination is of a different kind from intergenic recombination (Beadle, 1957), nor can gene conversion account for more than a small minority of intra-genic recombinants (for exceptions see Strickland, 1958).

On the other hand, localised negative interference, resulting in coincidence values of over 100 in some cases, is a normal feature of recombination in *Aspergillus* (Pritchard, 1955; and unpublished). This interference is only apparent between adjacent intervals the combined length of which is not more than a few tenths of a map unit.

The following hypothesis accounts satisfactorily for the data from *Aspergillus* and similar observations in other organisms. In any one cell the necessary conditions (effective pairing) for recombination are restricted to very short, discontinuously distributed segments of the genetic material. In a population of cells these segments will be distributed over the whole genome. Coincidence values greater than unity will be obtained for two adjacent intervals whose combined length is not much greater than the mean length of effectively paired segments since in most cells neither interval will be effectively paired, but when one interval is effectively paired, the effective pairing segment will usually cover both intervals, leading to a positive correlation between exchanges in the two intervals.

The recombination fraction between markers relatively far apart will depend on the frequency of effective pairing in the interval, and the frequency of exchanges within effectively paired segments. Interference operative over long intervals would result if effective pairing at one point influenced the probability of effective pairing elsewhere.

In *Aspergillus* preliminary data indicate that effective pairing segments are variable in length with a mean of about 0.3 map units. The mean number of exchanges per segment is between 1 and 2. The location of the segments is variable but may not be random.

PROKOFIEVA-BELGOVSKAYA, ALEXANDRA, in collaboration with V. N. BELIAEVA, D. D. ROMASHOV, N. N. ROTT, A. A. NEUFACH, and K. A. GOLOVINSKAYA. The Action of Ionizing Radiation on the Cell Nucleus in Early Development of Fishes. *Institute of Biophysics and Institute of Animal Morphology, Academy of Sciences of the USSR; Institute of Pond Fishery, RSFSR., Moscow, U.S.S.R.*

The problem of radiosensitivity of the early stages of fish development was investigated on the embryos of *Misgurnus fossilis*, *Acipenser stellatus*, *Salmo salar*, and *Cyprinus carpio*. Sperms, egg cells, and developing eggs through the stages from the first cleavage division to the late gastrula were treated with X-ray doses 50 r to 200,000 r. The radiosensitivity of the embryos was characterized by the degree of damage to cell nuclei (chromosome rearrangements), the mitotic index, survival percentage and percentage of monstrosities among the fry hatched. The degree of damage to the cell nuclei of the developing embryo depends upon the dose of radiation, the phase of mitotic cycle and the stage of development treated.

The parental chromosome sets in the blastomeres are spatially disconnected and differ up to the blastula stage as to the rate of cyclic changes during mitosis, the DNA content, the degree of basophily, and the ability to synthesise the nucleolus. The independence and the dissimilarity of cycles of development of the parental chromosomes lead to the specificity of radiation damage to the cell nuclei of blastomeres of the segmenting egg: doses up to 800 r affect preferentially one of the two chromosome sets of the nucleus.

The periodical changes in embryo radiosensitivity reflect the differences in radiosensitivity of the cell nucleus at different phases of mitosis during the period of synchronous cleavage divisions (till the early blastula stage). The degree of the damage to the cell nuclei, the number of nuclei affected and correspondingly the percentage of embryonic deaths increase progressively as the blastomeres pass from the interphase through the prophase to the metaphase. At the most sensitive phase of mitosis of the second cleavage division (2–4 blastomeres) a dose as small as 50 r causes a significant number of chromosome rearrangements (5.5% of bridges as contrasted with 0.8% in control). The early blastula stage is more sensitive than the late one.

Heavy doses of X-rays (10,000–50,000 r) were used in order to investigate the dependence of manifestation of the radiation damage upon the stage of development. When the embryo in its development reaches the period of slowed down and asynchronous divisions (early and middle blastula), its reaction on the treatment with heavy doses changes: irradiation before the early blastula stage leads to an arrest of development before the beginning of gastrulation, while a treatment

after the early blastula stage does not interfere with the process of gastrulation and the latter goes to the end. Correspondingly the time of the death of the embryo is shifted.

Different types of chromosome rearrangements persist through many cell generations and may be traced to the gastrula stage. There exists a clear dependence of the survival percentage and the frequency of appearance of monstrous embryos upon the degree of damage caused to the cell nuclei.

PUCK, THEODORE T. Genetic Studies on Somatic Mammalian Cells. *Department of Biophysics, University of Colorado Medical Center, Denver, Col., U.S.A.*

Simple methods for plating single mammalian cells on petri dishes so as to produce quantitative growth of discrete, macroscopic colonies have been developed. Clones of mammalian cells from a variety of normal and malignant, human and animal tissues have been isolated. In collaboration with Dr. J. H. Tjio, a rapid method of chromosome delineation, particularly suited to these preparations, has been developed and applied to cultured human cells with karyotypes containing 78 and 46 chromosomes and to opossum and Chinese hamster cells with 22 chromosomes. A defined medium with two purified protein constituents has been found which promotes quantitative growth of single cells of some types into colonies of any desired size. Naturally occurring mutant clones have been isolated with nutritional, virus-resistant, and chromosomal-morphologic markers which have remained stable throughout hundreds of generations. X-ray survival curves have been constructed, most of which approximate 2-hit character, and mean lethal doses for the cell reproductive function were measured and found to lie in the range of 60–150 r. A variety of evidence demonstrates that the destruction of cellular reproductive capacity by X-rays is due primarily to chromosomal damage. Several types of chromosome abnormalities are produced by X-irradiation even at and below doses of 25 r. Mutants with characteristic chromosomal constitution and nutritional behaviour have been isolated from X-ray survivors. Quantitative data on the radiogenetic damage to single cells will be presented and applied to interpretation of various mammalian radiobiological actions.

QUELCE-SALGADO, A. *See* FREIRE-MAIA, A.

QUINTANILHA, A. Mendelism and Michurinism. *Centro de Investigação Cientifica Algodoeira, Lisboa, Portugal.*

Michurinism is a biological theory elaborated by Lysenko in the name of Michurin and essentially characterized by the following principles: (1) denial of the existence of cellular material particles responsible for the transmission of hereditary characters; (2) negation of the continuity of the germinal plasma; (3) acceptance of the principle of inheritance of acquired characters; (4) denial of the existence of a struggle for life among the members of each biological population, responsible for the natural selection.

The author intends to prove that: Michurin himself was not a Michurinist in the sense of Lysenko—Michurin's most important horticultural successes were obtained

by the use of Mendelian methods; the materialist doctrine of the existence of cellular particles responsible for the transmission of hereditary characters is fully demonstrated; the continuity of germinal plasma is not a theory but a biological fact; the inheritance of acquired characters is in disagreement with any experiments intended to prove it; the rejection of the struggle for life as an explanation of natural selection is a misunderstanding—some Soviet biologists feared that, if we admit the existence of the struggle for life as a natural fact in animal societies, we have to accept the principle of competition among individuals of human societies as a normal and inevitable consequence.

RAMEL, CLAES O. M. O. Studies on Interchromosomal Effects of Inversions in *Drosophila melanogaster*. *Institute of Genetics, University of Stockholm, Stockholm, Sweden.*

The interchromosomal effect of inversions on crossing over seems to merit further invesigations as it might be a useful approach to the evaluation of the mechanism of crossing over. In this investigation the following points have been studied.

(1) A comparison was made of the effect of homozygous and heterozygous Muller 5 inversions in the X on crossing over in the second chromosome. The effect of heterozygous Muller 5 agreed with the results previously found for the interchromosomal effect of other X chromosome inversions on crossing over in the second chromosome, i.e., an increase chiefly in the vicinity of the centromere and an increase of multiple crossovers. Homozygous Muller 5 on the contrary showed only a slight effect compared to the control.

(2) In the Muller 5 chromosome the proximal heterochromatin has been displaced to the distal end. A study of the effect of autosomal inversions on crossing over in the distal end of homozygous Muller 5 was made. The increase of crossing over in this region in the presence of autosomal inversions did not show the characteristics of the corresponding increase in the distal part of a standard X chromosome but rather of the proximal part. This seems to indicate a direct effect of the inversions on the heterochromatic region.

(3) The influence of autosomal inversions on reversion of Bar did not give any evidence of clusters, pointing to gonial crossing over.

RAPER, JOHN R. The Genetics of Incompatibility in *Schizophyllum commune*. *Biological Laboratories, Harvard University, Cambridge, Mass., U.S.A.*

In the tetrapolar Hymenomycetes, sexual fertility of homokorgotic strains depends upon heterozygosity of two incompatible factors, *A* and *B*. A world-wide sample of 114 homokaryotic strains of *Schizophyllum commune* contained 96 distinct and interfertile *A* factors and 56 different *B* factors. Specific factors of each incompatibility series, *A* and *B*, appear to be equally frequent in the natural population and to be randomly distributed in respect to geographical and climatic location. These facts indicate 339 (5% limits of 562 and 216) *A* factors and 64 (5% limits of 79 and 53) *B* factors and an outcrossing efficiency of 98.15% in the natural population.

The *A* factor comprises two sub-units separable by crossing-over; recombination between component sub-units of paired *A* factors yields two new classes of factors

per cross that are (*a*) interfertile, (*b*) interfertile with both parental factors, and (*c*) often identifiable with factors collected from nature. In most pairings recombination frequency is between 3% and 6% at 23°C and is strongly affected by temperature. No distinction has been found in the action of the two sub-units: in respect to the *A* factor, strains are completely compatible when heterozygous for either sub-unit or heterozygous for both; incompatibility results only when both sub-units are homozygous.

Less extensive tests with the *B* factor show it to be comparable to the *A* factor in structure and in the inter-relationships of its components. Recombination between sub-units of the *B* factor has not been found in excess of 3%.

The nature of the individual sub-unit remains in doubt. Each may represent multiple alleles at a single locus or a number of very closely linked loci.

RASMUSEN, BENJAMIN A., CLYDE STORMONT, and YOSHIKO SUZUKI. Blood Groups in Sheep. *School of Veterinary Medicine, University of California, Davis, Calif., U.S.A.*

Several papers now in press and in preparation by the present authors are concerned with evidence for six genetic systems of blood groups in sheep in addition to the earlier recognized R-O system. These systems are named A, B, C, D, M, and X-Z, and the present note is intended to serve as a summary-statement of their essential features. The A system presently comprises a single pair of phenogroups, A as contrasted with no-A, and A antibodies are regularly engendered in isoimmunization of A-negative sheep with the blood of A-positive sheep. The B system which has its serologic and, presumably, genetic homology in the B system of cattle is, like B of cattle, very complex. So far, 52 B phenogroups have been identified in sheep, as compared to at least 164 in cattle, and doubtless many more will be discovered. The C system which has its serologic and, presumably, genetic homology in the C system of cattle is presently known to comprise three phenogroups: C, C_x, and no-C. With the development of new diagnostic reagents, it is possible that the C system of sheep might be expanded into a system which more closely parallels the complex C system of cattle. The D system, which is the only system studied by means of an agglutination rather than a hemolytic test, has two phenogroups, D and no-D. Three phenogroups (M, M_x, and no-M) are recognized in the M system; this system may be homologous to the S-U system of cattle. The X-Z system, with three phenotypes X, XZ, and Z, is, so far, much like the "two-allele" F-V system of cattle. Tabular data are presented, and other features of these six systems are discussed.

RASMUSSEN, INGE E. Persistence of Mutants in Laboratory Populations of *Drosophila melanogaster*. *Istituto di Genetica, Università di Pavia, Pavia, Italy.*

In a search for mutants of *Drosophila melanogaster* with heterotic effect, several autosomal recessives were tested in competition with the wild-type allele in experimental populations. Some mutants reached a stable frequency, probably due to the superiority of the heterozygote. So did *e*, *se*, *cd*, already known from the literature as having an advantage in the heterozygous condition, with equilibrium frequencies of .16, .25, .58, respectively. In addition *shv*, *bw*, *rsd* reached equilibrium frequencies of .54, .26, .32. The possibility of other explanations than overdominance

cannot be ruled out for some of these mutants, but seems quite unlikely for others. Such overdominant loci deserve special attention because of their effect on population variance and as a suitable material for a more analytical approach to the general problems of heterosis.

RATTENBURY, JOHN A. Cytogeobotanical Studies in the New Zealand Flora. *Department of Botany, University of Auckland, Auckland, New Zealand.*

The New Zealand angiosperm flora is said to contain a number of foreign elements. Plants with affinities with these areas are thought to have reached New Zealand at different times and to have remained subsequently to form a mixed flora in New Zealand. Most important of these affinities are an Antarctic element, with extensions to South America and Tasmania, and a Malayan element, extending northward through Melanesia.

Another hypothesis suggests that there has been a more or less continuous northward movement from an ancient temperate Antarctic flora into South America, Australasia and South Africa. Continuing northward from New Zealand some of these plants would eventually reach south-east Asia.

To test in part these alternative theories, viz.: whether there is a Malayan element in the New Zealand flora or a New Zealand element in the Malayan flora, a study of the incidence of polyploidy in plants belonging to the same genera in New Zealand and New Caledonia (which lies in the direct migration route between Malaya and New Zealand) is being undertaken.

Interpretation of such data is extremely difficult and conclusions may only safely be drawn on the basis of many chromosome counts where these point in a single direction. In the case of *Xeronema* and *Cordyline*, the diploids are in New Zealand and the polyploids lie to the north. In *Anthropodium*, diploids are present in New Zealand and New Caledonia while a tetraploid is present in New Zealand as well. Some forty to fifty additional species pairs will be examined shortly as a result of a trip to New Caledonia in February. These results will be presented in the paper.

RAVIN, A. W. Specificity in the Efficiency of Response by *Pneumococcus* to Different Transforming Agents. *Department of Biology, University of Rochester, Rochester, N. Y., U.S.A.*

A recipient strain of *Pneumococcus* treated briefly with deoxyribonucleic acid from a donor strain differing from it by a number of inherited characteristics may be transformed for any or all of these. However, not all the markers in a given transforming DNA preparation are acquired by the host population with the same efficiency. In recent experiments, a multiplying host population, deficient for capsule synthesis and sensitive to streptomycin and erythromycin, was treated for 15 min. (approximately one-half the generation time) with DNA extracted from a previously transformed clone of this strain, one that was encapsulated and resistant to 1 μ/ml. of erythromycin and to >100 μ/ml. of streptomycin. The treated population was then rapidly diluted in DNAase-containing medium and samples removed to inoculate parallel cultures such that an inoculum rarely contained more than one bacterium capable of giving rise to progeny transformed for a given characteristic. Each culture was later plated to determine the number of transformants of each type. The fraction

of parallel cultures containing transformants varied according to the marker. The marker affecting capsule synthesis appeared in the greatest number of parallel cultures, the marker controlling resistance to erythromycin was found almost as frequently, but the marker controlling resistance to streptomycin was found considerably less frequently. These differences were independent of the total transformation frequency, which varied with the degree of competence of the host population or with the length of time of exposure to transforming DNA in several otherwise similar experiments. These differences cannot be explained by any genetic heterogeneity in the donor population, for plate counts made on selective media just before the transforming DNA was extracted showed that every donor bacterium was encapsulated, streptomycin-resistant, and erythromycin-resistant. On the other hand, the extent of these differences is a function of the specific markers employed, for there are several different markers affecting various levels of antibiotic resistance and varying extents of capsule synthesis. A group of three markers for these characters can be selected, however, such that the observed differences in "uptake" are regularly large and significant.

The differences in "uptake" for the capsular, erythromycin and streptomycin markers are not due to differences in the times required for phenotypic expression of each of the markers. The time at which the parallel cultures are examined for transformants is well beyond the time required for maximal expression of each of the markers. These results will be discussed.

(These studies were supported by Research Grant E-727 awarded by the National Institute of Infectious and Allergic Diseases.)

RAY, CHARLES, JR. Tetraploidy in *Tetrahymena pyriformis*. *Emory University, Georgia, U.S.A.*

Three clones of *Tetrahymena pyriformis*, classified as mating types I, II, and III of variety 6, have been maintained in non-bacterial media since 1953. In 1956 we determined the chromosome number to be five pairs for each of the three clones. A re-study made in 1957 revealed that the micronucleus of one of the clones had become tetraploid. This spontaneous ploidy during asexual reproduction of the clone possibly came about through failure of the anaphase separation of duplicated chromosomes in the micronucleus. In the affected culture the tetraploid animals nearly replaced the diploid ones during the time between the origin of the ploidy and our discovery of it. No difference in body size or in macronuclear size of the tetraploids versus the diploids was detected. The micronuclei of the tetraploids were larger than those of the diploids. We noted in the tetraploid clone a marked reduction in the refractory period (from six to two hours) and pair formation within the clone (i.e., selfing) following suspension of the animals in distilled water. We had observed neither behavior in this clone before ploidy.

Using tetraploidy as a marker for the clone, we examined conjugants following a mixture of complementary mating types and found that the pairs formed first were between individuals of complementary mating types, while pairs formed about five hours later were mostly between individuals of the tetraploid clone (selfing). Using chromosome numbers of synkarya as evidence, we found that some synkarya had resulted from the fusion of sister pronuclei (i.e., cytogamy).

If conjugating pairs isolated for genetic analysis include instances of such deviations from a reciprocal exchange of pronuclei of complementary mating types, an

interpretation of the observed ratios might be difficult without an estimate of the extent of these different behaviors.

RAY, DAVID T. Sensitivity of Different Stages of the Eggs of *Mormoniella* to X-Rays. *Howard University, Washington, D.C., U.S.A.*

Female *Mormoniella* wasps were irradiated with varied dosages (500 r–1,000 r–1,500 r–2,000 r–2,500 r). They were placed with host pupae of the fly *Sarcophaga bullata* and transferred to new host pupae at frequent intervals. Eggs (laid at successive times after irradiation) had been irradiated at different meiotic stages. Eggs laid within 6 hours after irradiation have been X-rayed during the first meiotic metaphase. Egg counts were made using the damp chamber technique. Because of the parasitic nature of *Mormoniella* an accurate count of eggs can only be made by removing them from their host. The counted eggs must be placed in another host for further development. The new host pupae were first stung by sterile female wasps. A small aperture was made in the stung pupae and the eggs inserted within. The pupae were then placed in open mouth vials and suspended by metal racks over a saturated solution of NaCl in large covered jars, to ensure the correct humidity and discourage mold. The eggs were observed for hatching and development without being disturbed. Results indicate that while the number of eggs hatching decreased as expected with increase in X-ray treatment, the number of offspring from eggs laid during the first 6 hours decreased drastically. Only 8% of these eggs from females given 1,000 r developed as against 20% of eggs laid after 24 hours. No eggs developed from wasps given dosages above 1,500 r in this group. Reduction in the number of offspring from eggs laid in successive hours was not nearly as drastic. This seems to indicate a higher sensitivity of the first meiotic metaphase to irradiation, with the sensitivity decreasing in the earlier meiotic and premeiotic stages. (This work was supported under National Science Foundation Grant NSF G–2587.)

RAY, DAVID T. *See* WHITING, P. W.

REED, T. EDWARD. Methods and Problems in Estimating Relative Fitness in Man: Examples from a Study of Huntington's Chorea. *Department of Human Genetics, University of Michigan, Ann Arbor, Mich., U.S.A.*

Relative fitness (W) of individuals with a specific genetic trait has customarily been defined, and measured, as the ratio of the mean number of children born to the trait-bearers to the corresponding mean of the normal sibs of the trait-bearers. It is assumed, but is rarely demonstrated, that the normal sibs give a valid estimate of the fertility of the general population. This study reports an example of a significant difference in fertility between normal sibs and the general population and makes suggestions for estimating relative fitness.

An extensive survey of Huntington's chorea (H.C.) in Michigan (U.S.A.) provided an opportunity to estimate relative fitness for a dominant trait whose W is near unity. Using individuals who had completed their reproduction, it was found that the relative fitness of heterozygotes for the gene for H.C. was 1.03 ± 0.11 if the

conventional sib comparison was made. Comparison with appropriate age-specific fertility data of the U.S. Census, however, revealed that these normal sibs were significantly less fertile than the females of Michigan. Comparing heterozygotes with these normal Michigan females gives a W of 0.79 ± 0.12.

Because the ratio of mean numbers of children ever born does not necessarily express the relative rates of growth of the two classes being compared, a more precise definition of relative fitness is offered. This makes use of the parental age distribution as well as the mean number of livebirths ever born per newborn individual.

Other aspects of estimating relative fitness are also considered. It is strongly urged that, when possible, W be estimated directly as the ratio of (a) mean number of children ever born to trait-bearers to (b) the corresponding mean, comparable in date and age range, for the general population.

REEVE, E. C. R. The Induction, by X-Rays, of Mutations Affecting Quantitative Characters in *Drosophila melanogaster*. *Institute of Animal Genetics, University of Edinburgh, Edinburgh, Scotland.*

Our ignorance of the rate at which mutations affecting different quantitative characters arise spontaneously or in response to mutagenic agencies forms one of the biggest gaps in our knowledge of population genetics, and the present paper will describe some work in this field, using a method which enables the mutational variance of several characters to be measured at the same time.

By a suitable crossing scheme with *Drosophila melanogaster* it is possible to make standard irradiated third chromosomes homozygous in a genetic background identical with the inbred line providing the third chromosomes. A number of such strains have been produced, using an X-ray dosage of 4,000 r, and both the homozygous strains and intercrosses between them have been raised under standard conditions.

Wing and thorax length and the numbers of sternite and sternopleural hairs were estimated on these strains and crosses, and also on control strains obtained by the same mating scheme without irradiation. Strains in which third chromosomes from a wild stock were made homozygous in the same inbred genetic background were also studied, to give a standard against which the radiation-induced genetic variance could be compared.

The results of these experiments will be discussed, but one point of interest which emerged may be noted here. The environmental variance in body size among individuals of the same genotype was, on the average, greater for the homozygous strains than for their intercrosses. This suggests that the third-chromosome heterozygosity induced by 4,000 r of X-rays was sufficient to increase the environmental stability of the intercrosses over that of the homozygotes.

REMPEL, WILLIAM E. *See* SUMPTION, LAVON J.

RETHORE, MARIE ODILE. *See* LEJEUNE, JERÔME.

RICHTER, ALAN A. Recombination Analysis of Mating Type in *Escherichia coli* K12. *Department of Genetics, University of Wisconsin, Madison, Wis., U.S.A.*

An F– strain, ♀ 3, was previously reported to be convertible, upon exposure to F+ cells, to the Hfr_3 mating type, here termed ♂ 3, which displays high frequency of segregation for markers in region 3 (Th M Xyl S). It was shown that the ♂ 3 phenotype depends upon the presence of the transmissible agent F and a gene Hfr_3+ which does not recombine with a marker *Mal*$_5$. From a cross of ♂ 2 (Hayes Hfr strain) × ♀ 3, four mating genotypes are recovered, the two parental types, standard F– (convertible to standard F+), and a type referred to as compound male. The same cross demonstrates that the ability to yield infectious F agent does not recombine with the ♂ 2 quality. One may therefore postulate that the F agent of ♂ 2 resides at a locus Hfr_2, thereby determining the ♂ 2 phenotype. Hfr_2 is defined by its position in the following sequence: Gal Lac P L T Hfr_2 Hfr_3 Th M Xyl S. The compound male type has the following properties in common with ♂ 2. (1) In crosses with F–, it displays high frequency of segregation for markers in region 2 (T L P Lac Gal) and low frequency of segregation for markers outside region 2(Hfr_2 Mal_5 Th M Xyl S). (2) It is stable as shown by the fact that the vast majority of progeny from crosses × F– are F–. (3) The ♂ quality recombines with Mal_5. It is possible to distinguish the compound male from ♂ 2 by the fact that Hfr_3 segregates in crosses of compound male × F–. The new mating type therefore carries both the determinant of the ♂ 2 property (F present at Hfr_2) and the determinant of the ♀ 3 property (Hfr_3+).

RICK, CHARLES M. Disturbed Segregation in Progenies of *Lycopersicon esculentum* × *L. chilense*. *University of California, Davis, Calif., U.S.A.*

Several F_1 hybrids were obtained between *Lycopersicon esculentum* homozygous for markers (*a, c, d,* l_1) on four chromosomes and a stock of *L. chilense* that possesses the respective dominant alleles. Meiosis, including pachytene pairing, appears normal, and about half the pollen is aborted. Since the F_1 hybrids exhibit the strict self-incompatibility of the *chilense* parent, F_2 progenies were obtainable only by intercrossing intercompatible hybrids. A first BC generation was obtained by using the recessive *esculentum* stock as female parent.

Recessive homozygotes are generally deficient in F_2 and in excess in BC. The degree of deviation from *esculentum* controls varies according to the gene, highly significant departures having been observed for *d* and l_1, and less marked discrepancies for *a* and *c* in both F_2 and BC. The deficiencies in F_2 are in accord with a shift toward *L. chilense* observed in distributions of quantitative species-differentiating characters. Significant heterogeneity exists between BC progenies for both *a* and l_1, but not for *c* and *d*. Differential survival is suspected to have caused this heterogeneity, since germination is variable and never exceeds 25%. A general but non-significant tendency was observed toward association between parental character combinations in F_2. A similar but less consistent trend appeared in BC. Segregations in several second BC families closely approached normal.

The opposing deviations of BC and F_2 discount imperfect penetrance and differential survival of gametes during maturation as the sole causes of the disturbed segregations. Differential pollen tube growth and/or zygotic elimination could be

completely responsible, the latter being suggested by the greatly reduced seed fertility.

(Support for this work by grant No. G–3355 of the National Science Foundation is gratefully acknowledged.)

Rieger, Rigomar, and Arnd Michaelis. The Induction of Automutagenic Substances by Submersion of *Vicia faba* Seeds in Tap Water. *Institut für Kulturpflanzenforschung der Deutschen Akademie der Wissenschaften zu Berlin in Gatersleben, Kreis Aschersleben, Germany.*

Seeds of *Vicia faba* L. var. *minor* ($2n = 12$) were submerged in tap water and left to soak there for 12, 24, 48, 72 and 96 hours. After submersion and recovery for 48 to 144 hours in petri dishes, root tips were fixed at intervals of 24 hours. The presoaking of the seeds led to a remarkable increase of the spontaneous chromosomal aberration rate. Up to 65% of all cells in the root tip meristem showed fragments, chromatid translocations and triradials after 72 hours of presoaking and up to 12% of all chromosomes showed breaks or participated in chromatid reunions. Submersion for 72 hours called forth the maximal effect, and was not increased by presoaking for 96 hours. Submersion of seeds for 12 hours only caused a statistically significant increase of chromosomal aberrations in relation to the controls. The maximum of aberrations was found after a recovery period of 120 hours; afterwards the aberration rate dropped and the aberration spectrum changed because of cell selection. All reunions occurred on chromatidal basis apart from the triradials. The highest number of breaks per cell was 10. The distribution of breaks between the small m- and the large SAT-chromosomes was not random (theoretical expectation 5:2); more breaks appeared in the small chromosomes (actual relation m:SAT = 5:1.1). The breaks were localized in a few probably heterochromatic regions.

To exclude the possibility that the effect of submersion was really caused by bacterial products, parallel experiments were done with a N_2-atmosphere. These experiments showed that the increase in aberration rate in both cases was caused by metabolic changes induced by anaerobiosis. Probably the changed metabolic conditions led to the formation or accumulation of automutagenic substances.

The leaves of the young seedlings growing from seeds submerged for 48 to 96 hours were characterized by growth abnormalities and variegation caused by chromosome mutations.

Our experiments show that anaerobiosis under special circumstances is to be viewed as one of those factors which lead to spontaneous mutations because one must reckon with periods of partial anaerobiosis under natural conditions also. The effect of anaerobiosis found in *Vicia* according to preliminary observations is not reproducible in barley probably because of other reserve substances in the barley seeds not found in *Vicia.*

Riley, Herbert Parkes. Karyotypes of Some South African Species of Plants. *University of Kentucky, Lexington, Ky., U.S.A.*

Chromosome counts were made from the root tips of germinated seeds of 55 South African species of plants from 47 genera and 21 families. In an attempt to bring out

secondary constrictions and heterochromatic regions, various chemical compounds were used as pretreatments or as additions to the fixative, including bromonaphthalene, aesculin, chloral hydrate, 8-oxy-quinoline, coumarin and paradichlorbenzene. Chromosome counts were confirmed for 10 species previously studied by various investigators. Chromosome numbers and karyotypes were obtained from the following species which belong to genera in which chromosomes have apparently not been studied previously: *Clematopsis stanleyi* (Family Ranunculaceae), *Pollichia campestris* (Caryophyllaceae), *Acrodon bellidiflorus* (Ficoidaceae), *Adansonia digitata* (Bombacaceae), *Triaspis nelsonii* (Malpighiaceae), *Schotia brachypetala* (Caesalpiniaceae), *Baphia obovata* and *Bolusanthus speciosus* (Papilionaceae), *Berchemia speciosus* (Rhamnaceae), *Melianthus comosus* and *M. minor* (Melianthaceae), *Adenium multiflorum* (Apocynaceae), *Hoodia Burkei* and *Pachycarpus schinzianus* (Asclepiadaceae), *Nautochilus labiatus* (Labiatae), and *Aristea thyrsiflora* and *Dietes prolongata* (Iridaceae). Chromosome numbers and karyotypes were determined for the following species in which chromosomes have apparently not been previously studied, although chromosome counts are on record for other species of the respective genera: *Glottiphyllum muirii*, *Delosperma mahonii*, *D. sutherlandii*, and *Dorotheanthus gramineus* (Ficoidaceae), *Momordica clematidea* (Cucurbitaceae), *Terminalia phanerophilia* (Combretaceae), *Cassia granitica* var. *abbreviata* (Caesalpiniaceae), *Dichrostachys nyassana* and *Acacia pallens* (Mimosaceae), *Erythrina lysistemon* (Papilionaceae), *Cissus lanigerus* (Ampelidaceae), *Carissa grandiflora* (Apocynaceae), *Aster capensis*, *Felicia rotundifolia* and *Vernonia glabra* (Compositae), *Ipomoea albivenia* (Convolvulaceae), *Coleus barbatus*, *C. pentheri*, *Plectranthus myrianthus*, *Pycnostachys reticulata* and *P. urticifolia* (Labiatae), *Chlorophytum longipedunculatum*, *Eucomis regia*, and *Gloriosa virescens* (Liliaceae), and *Babiana bainesii*, *Moraea polystachya*, *Watsonia ardernei* and *W. fourcadei* (Iridaceae). Secondary constrictions were observed in the chromosomes of many of the species.

RILEY, RALPH. Chromosome Pairing and Haploids in Wheat. *Plant Breeding Institute, Cambridge, England.*

Four kinds of haploid have been obtained in *Triticum vulgare* var. Holdfast (6x = 42) from lines involved in making additions and substitutions of single pairs of rye chromosomes. (1) Euhaploids with 21 chromosomes had the complete haploid chromosome set of wheat. (2) Two types of 22-chromosome addition haploid had the haploid complement of wheat plus one rye chromosome. (3) Three types of 20-chromosome nulli-haploid had the haploid set of wheat chromosomes minus one. (4) One 21-chromosome substitution haploid had 20 chromosomes of the haploid set of wheat plus one rye chromosome. At first metaphase of meiosis in euhaploids, in the addition haploids, and in one of the nulli-haploids, there were mean bivalent frequencies of 1.0 to 1.7 per cell, and there was little difference between the three classes. Nulli-haploids of the second type, and the substitution haploid with the same 20 wheat chromosomes, had fewer bivalents than euhaploids (mean 0.7 to 0.9 per cell), suggesting that the missing chromosome often participated in bivalent formation in euhaploids. However, in the third nulli-haploid there was a mean of 4.2 bivalents and 0.8 trivalents per cell. Cells with three trivalents were not uncommon and 29% of cells had five to seven bivalents, in contrast to the other haploids examined in which trivalents were very rare and not more than four bivalents per

cell were formed. Further, in this nulli-haploid 20% of the total bivalents were rings, whereas no more than 4% were rings in the other haploids. It seems, therefore, that the chromosome deficient in this nulli-haploid carries a gene which reduces pairing, so restricting association between homologous chromosomes. Such a gene would have selective value in hexaploid wheat for the effect which cytologically diploid behaviour would have on fertility and genetic stability.

The evolutionary significance of this situation and its potential value in wheat breeding, especially in the introduction of alien genes, will be considered, reference being made to the behaviour of nullisomics and alien chromosome substitutions.

RIPPON, JOHN W. and GEORGE H. SCHERR. Induced Dimorphism in Pathogenic Fungi. *Department of Microbiology, College of Medicine, University of Illinois, Chicago, Ill., U.S.A.*

Based on the concept that pathogenicity among the deep and superficial mycoses is, to a major degree, a direct function or limitation of the capacity of the organism to grow in a yeast phase, studies were undertaken in which transformation of mycelial (M) to yeast (Y) phase for the superficial dermatophytes has been attempted along with the transformation of M to Y phase growth in *Histoplasma capsulatum* at temperatures less than 37°C. Utilizing the gradient plate technique, cultures of *Microsporum audouini*, *Trichophyton rubrum*, and *Cladosporium mansoni* were subjected to increasing concentrations of cysteine at temperatures of 25°, 30°, and 37°C. *M. audouini* and more particularly *T. rubrum* growing at 37° in the areas of higher cysteine concentration appeared yeast-like. *C. mansoni*, which normally has some yeast-like character when grown at room temperature, appeared nearly 100% as a budding yeast. Combinations of temperature and cysteine radically altered pigment production as well as the morphology of the dermatophytes. Ether-acetone extracts of *Saccharomyces cerevisiae* were tested for their ability to induce M to Y transformation of *H. capsulatum* at temperatures other than 37°C. The results indicated activity of some fractions to convert mycelium to yeast phase growth at 25°C. The ramifications of these data with reference to the pathogenesis of these diseases will be discussed.

(This report is part of a study supported by Grant No. E–1651 from the U.S. Department of Health, Education, and Welfare, National Institutes of Health.)

RIS, HANS. Fine Structure of the Nucleus during Spermiogenesis. *Department of Zoology, University of Wisconsin, Madison, Wis., U.S.A.*

The study of chromosomes and interphase nuclei with the electron microscope has suggested that chromosomes consist of microfibrils. Fibrils about 100 Å thick seem to be a universal component of chromosomes and the smallest structural unit undisputably resolved in somatic nuclei. Generally these fibrils are associated in pairs, forming a next higher unit about 200 Å thick and in prophase chromosomes these are again organized by two's into units about 500 Å thick. In addition to these fibrils a complex larger structure has been described in meiotic chromosomes of animals, the so-called core or central structure (Moses, Fawcett). We have been interested especially in these two problems: (1) what is the relation of these fibrils

to the known chemical constituents of chromosomes (DNA, RNA, basic proteins, residual proteins); (2) what is the constant and persisting structure in chromosomes, the material of genetic continuity in contrast to structures which appear only under specific conditions? In a study of the nucleus during spermiogenesis one would expect the chromosomal material to be reduced to the bare essentials for genetic continuity. Chemical studies show that the composition of the nucleus undergoes profound changes during spermiogenesis. What happens to the chromosome fibrils during this process? Electron micrographs will be shown illustrating the nuclear changes during spermiogenesis of several insects (grasshoppers, roaches), cephalopods (sepia, octopus), and vertebrates (minnow, rooster, rat). In all these we found that the 100 Å fibrils seen in early spermatid nuclei split up into fibrils, about 40 Å thick, which then fuse into larger complexes in which the individual fibrils are no longer visible. Finally the entire sperm nucleus becomes uniformly dense and homogeneous. The specific way in which the 40 Å fibrils aggregate, however, and the orientation of the fibrils in the sperm nucleus vary greatly. In some species the fibrils line up side by side into complexely folded sheets, in others they fuse into straight or much twisted bundles which gradually combine into larger complexes. X-ray diffraction studies on sperm nuclei have shown that DNA molecules are oriented parallel to the long axis of the sperm (Wilkins). During spermiogenesis the microfibrils straighten out and line up in the same way. This suggests that the DNA is arranged parallel to the long axis of the chromosomal microfibrils.

RIZET, GEORGES. Un nouvel Ascomycète intéressant pour les généticiens, *Ascobolus immersus*. *Faculté des Sciences de Paris, Laboratoire de Génétique, Paris, France.*

Le genre *Ascobolus* a déjà fait l'objet de diverses études génétiques et plusieurs espèces, *A. immersus*, *A. stercorarius*, ont permis d'obtenir quelques mutants dont les caractères sont visibles sur les ascospores. Celles-ci sont projetées à maturité et peuvent être reçues par groupe de huit sur un milieu transparent où elles sont facilement observables. On peut ainsi, très rapidement, observer des milliers d'asques. *A. immersus*, de plus, possède des ascospores énormes dont l'isolement est d'une grande facilité.

Plus de quatre vingt mutants, apparus spontanément et intéressant les caractéres de l'ascospore ont été isolés. Il s'agit le plus souvent d'ascospores peu pigmentées ou totalement incolores, mais aussi d'ascospores à membrane rugueuse ou enfin d'ascospores sur lesquelles le pigment se dépose sous forme de gouttelettes plus ou moins grosses et plus ou moins nombreuses. Nous avons donc pu observer des ségrégations extraordinairement variées, visibles immédiatement.

Plus de la moitié des gènes ainsi définis appartiennent au même groupe de linkage. Tous les degrés de linkage ont pu être observés et il devient très facile de faire l'analyse du crossing-over et des variations de sa fréquence. De très nombreuses anomalies de ségrégation décrites sous le vocable de conversion ont été vues: leur fréquence est variable selon les gènes envisagés et selon le milieu. Près des deux tiers des gènes se répartissent en une dizaine de séries d'allèles; soit d'homo-allèles avec fréquence de réversion pratiquement nulle, soit d'hétéroallèles avec réversions plus nombreuses et quantitativement mesurables par l'analyse directe des tétrades.

Ce matériel se révèle donc bien adapté à l'étude de problèmes variés.

Rizki, M. T. M. Factors Influencing Haemocyte Transformation in Normal and Tumorous Larvae of *Drosophila melanogaster*. *Reed College, Portland, Oregon, U.S.A.*

It was previously demonstrated that there are two main classes of blood cells in larvae of *Drosophila melanogaster* (Ore-R strain), crystal cells and plasmatocytes. The plasmatocytes are spherical cells which at the time of formation of the white puparium develop filamentous cytoplasmic extensions. This morphological transformation continues during early pupation until extremely flattened cells are formed. These flattened cells are the lamellocyte variants. In the tumorous strains, tu^{w} and mt^{A}, this mass transformation of plasmatocytes to lamellocytes occurs at the second molt and in the early third instar. The lamellocyte aggregates form tumorous masses which later become melanized. This precocious transformation of blood cells in these tumorous strains suggested an upset in the normal pattern of metamorphosis of the haemocytes.

The influence of humoral centers such as the ring gland and brain on metamorphosis has been established in *Drosophila* and other insects by several authors. A causal analysis of transformation of the larval blood cells of *D. melanogaster* has been attempted by a series of ligature experiments. Early third instar larvae (64–66 hours at 24°C) of the Ore-R strain have been ligatured so that the brain and ring gland humoral centers (BRH) are excluded from the posterior part of the body. Control larvae were ligatured slightly anterior to those of the experimental group in such a way that the BRH was included in the posterior part of the body. After 24 hours blood samples were examined from the posterior region of the ligatured larvae. Exclusion of the influence of BRH results in mass transformation of plasmatocytes to lamellocytes, whereas this phenomenon is not noted when the plasmatocytes are exposed to the influence of BRH. Humoral factors play a significant role in the transformation of the plasmatocytes, and these experiments support the hypothesis that the physiological syndrome controlled by the tumor genes includes an upset of the humoral mechanism. (Work supported by National Science Grant G–3381 and Public Health Service Grant RG–5285.)

Roberts, J. A. Fraser. Some Further Observations on Associations between Blood Groups and Disease. *Clinical Genetics Research Unit, Institute of Child Health, Hospital for Sick Children, London, England.*

A number of mental hospitals collaborated in a survey organized by the Royal Medico-Psychological Association, grouping newly admitted patients during the calendar year 1957. The total number is about 20,000. The forms are now being coded and the cards punched. It is hoped to present a preliminary account of the results.

In collaboration with Professor Ian Aird and his Department data have been collected on three conditions. In 600 cases of cancer of the pancreas there was an excess of Blood Group A, significant at the 5% level. If confirmed, this will be another example of a positive result with a disease associated with the upper part of the gastro-intestinal tract. In 600 cases of cancer of the oesophagus a relative incidence in Group A of 1.1 as against 1 in Group O appeared. This would equally fit a negative result or the 1.2 so far found in cancer of the stomach. It illustrates the large numbers needed in this kind of investigation. But 400 cases of chromo-

phobe adenoma of the pituitary do not show the excess of Group O which was found by Professor Mayr and his colleagues at Boston. It would be interesting to discuss possible reasons for different findings in different regions in this and other conditions.

ROBERTS, MARGARET R., CAROLYN F.-R. DISSOSWAY, and HAROLD H. PLOUGH. Evidence for Linear Order of Transducible Loci and Prophage Sites in *Salmonella typhimurium*. *Amherst College, Amherst, Mass., U.S.A.*

Although linkage of units within loci in *Salmonella typhimurium* has been shown by Demerec and his associates, there is no current explanation of differences in frequency of transduction from one locus to another. Yet the transductions for each locus are fairly constant with each strain at similar multiplicities.

We have been studying transduction on a triple auxotroph of strain 533 by phage derived from 549 cells, using new plating methods and a cleared indicator strain, TC. The 549 cells are now recognized to be doubly lysogenic for phages 549t and 549m. Using the loci Histidine (H^-), Tryptophane (T^-) and Leucine (L^-) on a sub-strain of 533, transduction frequencies were determined with phages from: (1) the original lysogenic strain, 549; (2) this same phage grown on 533 host cells. The transduction frequencies per 10^8 cells at approximately the same multiplicities are shown in the following experimental results: 549(549t) + 533 $H^-T^-L^-$ gave 27.6 H^+, 13.8 T^+, 2.6 L^+; 533(549t) + 533 $H^-T^-L^-$ gave 73.0 H^+, 217.3 T^+, 7.4 L^+.

These results may be explained by assuming an arrangement of loci in a constant linear order, with the frequency of transduction in inverse relation to the proximity of any locus to the transducing prophage site. Differences in frequency of lysogenization of the three types of transduced cells in each series are directly related if the principal prophage sites are transposed. Actually the prophage sites for phages 549t and 549m have apparently been reversed in 549 and 533 strands.

The following indicates the map order for the 549 and 533 series (prophage sites in parentheses): 549 (*549m*) H^+ T^+ (*549t*) L^+; 533 (*549t*) H^+ T^+ (*549m*) L^+. These arrangements appear to be the only ones which satisfy the requirements of our available data.

The order of gene loci in *Salmonella* is interpretable in much the same way as in chromosome maps for higher forms, with transductions, like crossing over, introducing new segments in inverse relation to the proximity of the genes to the prophage site.

ROBERTSON, FORBES W. Cell Size and Number in Relation to Heterosis and Size Variation in *Drosophila*. *Institute of Animal Genetics, University of Edinburgh, Edinburgh, Scotland.*

Wing area of *Drosophila* is highly correlated with the size of the fly. By estimating cell density in the wing membrane, variation of wing size due to different kinds of genetic and environmental cause has been found associated with characteristic changes in the cell size and number relations. Culture at different temperatures affects only cell size. By rearing larvae on chemically defined, aseptic media, equivalent reduction of size may be effected by altering the level of specific nutrients.

Different treatments lead to striking contrasts in cell size and number; frequently all the reduction in wing can be attributed to change in a single component.

Comparisons of variance between wild populations and genetically uniform crosses between inbred lines indicate substantial genetic variation of cell size, which can be divided into a major part which is uncorrelated and a smaller fraction which is correlated with wing size. The former presents an aspect of the regulation of wing size, since there is a strong negative correlation between cell size and number. Selection experiments suggest that such variation is largely additive in behaviour. Response to selection for large and small size depends, at least in the early stages, on change in cell number. But crosses between selected strains, between inbred lines and also between geographical races show heterosis or a positive deviation from intermediacy in wing and cell area. Wing and cell area deviate about equally on a log scale; so the heterosis is expressed via cell size.

Evidence from genetic and environmental variation suggests that the factors which influence cell size and number are partly independent in action and show a different genetic behaviour in terms of additive and non-additive effects. The data, which are relevant to discussion of intra-population heterosis, asymmetry in selection response, inbreeding decline and gene-environment interaction, also illustrate the advantages of going beyond a purely numerical description of a quantitative "character."

ROBINSON, H. F., C. C. COCKERHAM, and R. H. MOLL. Genetic and Genotype-Environmental Interaction Variances in Maize. *North Carolina State College, Raleigh, N.C., U.S.A.*

The variation in quantitative characteristics as reflected in phenotypic expression is a function of genotype and the environment in which it is produced. Phenotypic expression, the basis on which the breeder makes his choice, varies not only with the genotype composition and environmental effects which may be operating independently but with identical genotypes responding differently in different environments. The magnitude of genotype-environmental interactions, relative to the genotypic variance, can be of major concern to the breeder where the soundness of decisions on procedures to follow relates to the usable genetic variances.

The study reported here represents an attempt to obtain measures of genotype-environmental interaction variances that may be contributing to "biases" in estimates of genetic variances in maize populations. A series of half-sib families were produced for testing over a variety of locations and series of years. Five locations are used to represent the population of environmental effects that may be contributing to genotypic differences encountered at different places within a region of adaptation. The genotype-environmental interactions are measured in terms of estimated interaction variances of genotypes $\times$ years, genotypes $\times$ locations and genotypes $\times$ locations $\times$ years. Groupings of locations over the region of locations for which inferences are to be made enables an examination of the contributions of sub-region environmental fluctuations to genotype-environmental interactions. The findings in this study are compared with estimates of genetic and genotype environmental interaction variances available from other studies with maize.

ROBINSON, H. F. *See* MATZINGER, D. F.

ROBSON, E. B. *See* HARRIS, H.

ROBSON, H. N. *See* BENNETT, J. H.

ROLLINS, W. C., and K. A. WAGNON. Genotype Environment Interaction in a Beef Cattle Herd. *University of California, Davis, Calif., U.S.A.*

A genetic analysis has been made of 335 long yearling heifer weights collected in two experimental range beef cattle herds, A and B, of similar breeding that were managed alike except that in herd A the heifers were supplemented to promote continuous growth during the fall and winter (post weaning) when the range was nutritionally deficient, while the heifers in herd B were not supplemented and usually lost weight during the period in question. Both groups made rapid gains during the subsequent period of good pasture. The heifers were long yearlings (about 20 months of age) at the end of the spring pasture season.

Heritability of weaning weight (weaning was at eight months of age) was estimated to be .36 for herd A and .42 for herd B. Heritability of long yearling weight was estimated to be .42 for herd A and —.23 for herd B. For gain from weaning to long yearling age, heritability was estimated to be .25 for herd A and —.04 for herd B. A genetic correlation of .74 and an environmental correlation of .62 was found between weaning weight and long yearling weight in herd A, while for herd B these were zero and unity, respectively.

Since in paternal half-sib estimates of heritability and genetic correlation dominance effects occur statistically in the term for random environmental effects, it is felt that these estimates considered jointly suggest a greater importance for heterozygosity and a lesser importance of additively genetic effects under B herd than under A herd conditions.

ROMASHOV, D. D. *See* PROKOFIEVA-BELGOVSKAYA, ALEXANDRA.

ROPER, J. A. Genetic Analysis of Cytoplasmically Determined Variation in *Aspergillus nidulans*. *Department of Genetics, University of Glasgow, Glasgow, Scotland.*

Strains of *Aspergillus nidulans* carrying nutritional markers and also resistant (single gene determined) to acriflavine undergo irreversible changes after serial subculture on medium with acriflavine. Controls without acriflavine are unchanged. Three such modified strains have so far been studied. They bear few or no conidia and are described as "mycelial." They are stable on repeated subculture, by hyphae or conidia, on any medium so far tested, with or without acriflavine. The mycelial condition can be transmitted to other genotypes (with or without a mutant allele for acriflavine resistance) by heterokaryosis. From balanced heterokaryons between strains A mycelial and B non-mycelial (where A and B represent differently marked nuclei) one recovers the types A mycelial, B non-mycelial and B mycelial but never

the type A non-mycelial. Nucleo-cytoplasmic interactions are pronounced and each mycelial cytoplasm gives a wide range of phenotypes in combination with different genotypes.

The three original mycelial types have been shown to be cytoplasmically different by combining each cytoplasm with a common genotype; the three strains so obtained were morphologically quite distinct. Heterokaryons formed between pairs of strains, each with a different mutant cytoplasm, did not restore the non-mycelial phenotype.

Balanced heterokaryons (and heterozygous diploids derived from them) between a mycelial and a non-mycelial type are almost normal in phenotype, suggesting that the mycelial condition is little expressed in a prototroph. However, mitotic recombination in such diploids gives types of which a proportion are mycelial. At mitotic recombination the determination of a mycelial versus a non-mycelial phenotype appears to be correlated with the particular genotype produced by crossing over or haploidization.

Crosses between a mycelial and a non-mycelial give both mycelial and non-mycelial types. Preliminary results suggest that segregation of "mycelial" at meiosis is irregular.

Ross, James G., Clifford J. Franzke, and Mary E. Sanders. The Immediate Induction of Homozygous Diploid Mutants in Sorghum. *Agronomy Department, South Dakota State College, Brookings, S.D., U.S.A.*

True-breeding, diploid, mutant plants arise from colchicine treatment of sorghum seedlings of lines Experimental 1 and Experimental 3. It was proposed that in Experimental 3 the mutant condition might arise through concentrations of chromatin from ancestors from which this line was derived, and that the true-breeding diploid condition might arise by a somatic reductional division of the chromosomes with subsequent restoration to the diploid number.

The first part of the hypothesis was tested by observing meiotic pairing relationships in true-breeding mutants and in F_1 hybrids between mutants and their untreated full sibs. No irregularities were observed indicating no major relocation of chromatin in the mutants. Genetic analyses of F_2 populations from these crosses provided evidence that genotypic changes resulted from point mutations. Study of an F_3 population from a cross between one mutant and its untreated full sib gave an estimate of at least 13 changed genes. Induction of a lineal series of diploid mutants (all showing normal meiosis) by colchicine treatment of each successive new mutant furnished additional evidence for the mutagenic action of colchicine.

To test the second part of this hypothesis, seedlings with chromosome markers (recessive genes and reciprocal translocations) in the heterozygous condition were treated with colchicine. The hypothesis is supported by the appearance of homozygous shoots after colchicine treatment of F_1 flax plants heterozygous for known genes. Some sorghum lines fail to produce obvious true-breeding diploid mutants following colchicine treatment, and lines known to give mutants do not always do so. Failures to find homozygous shoots after treatment of heterozygous materials may also be due to either an unfavorable internal (genotype) or external environment. The effects of water availability, nutrition, light and temperature during treatment are being studied. The original mutants arose in an unshaded greenhouse; recent results show that diploid mutants can also be obtained by colchicine treatment under infrared light.

ROTHENBUHLER, WALTER C. Genetics of a Behavior Difference in Honey Bees. *Department of Zoology and Entomology, Iowa State College, Ames, Iowa, U.S.A.*

Honey bees (*Apis mellifera* L.), living in highly organized social units, show an extremely rich display of behavior. Behavior patterns are evident in the gathering of nectar, pollen, and water, the sharing of food, the building of comb, the care of the brood and the broodnest, the casting of swarms, the use of propolis in the hive, the defence of the colony, and the transmission of information about food sources by the well-known dances. Furthermore, different strains and races show characteristic differences in some of these behavior patterns. Since artificial insemination makes control of matings possible, genetics of honey-bee behavior now can be studied readily.

One of the mechanisms by which honey bees resist the larval disease American foulbrood (causative organism: *Bacillus larvae* White) has been observed to be behavioristic. The present study revealed that one selected, inbred, resistant line of bees removed all of the diseased brood from the test combs prior to the sixteenth day following inoculation on the first day of larval life. Peak removal occurred usually on the eighth to tenth days. The selected, inbred, susceptible line allowed most of the diseased brood to remain in the test combs throughout the experiments which ended on the sixteenth day.

Reciprocal crosses of these two lines produced an F_1 generation which allowed diseased larvae to remain in the broodnest in the same way as the susceptible line. Hence, resistant or hygienic behavior appears to be due to recessive heredity. Experiments, now in progress, with back-crosses of the F_1 generation are expected to yield information on the number of genetic elements involved in this difference in behavior. (This investigation was supported in part by a research grant E599(C7) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service.)

ROTT, N. N. *See* PROKOFIEVA-BELGOVSKAYA, ALEXANDRA.

ROWLANDS, D. G. *See* GRIFFITHS, D. J.

RUDKIN, G. T., and S. CORLETTE. Disproportionate Synthesis of DNA in Polytene Chromosomes. *Institute for Cancer Research, Philadelphia, Pennsylvania, U.S.A.*

Puff regions in polytene chromosomes of Diptera are thought to be regions of high gene activity. Breuer and Pavan's belief that deoxyribonucleic acid is accumulated in a puff region in *Rhynchosciara angelae* was confirmed by us in a preliminary report last year. In the present paper we shall present additional evidence pointing in the same direction. Quantitative estimates are made by measuring the absorption of

monochromatic ultraviolet radiation in a chromosome region, and taking advantage of the absorption bands of nucleoproteins. The analysis of a chromosome into nucleic acid types and protein is carried out by measuring the change in absorption brought about by treating the chromosome with ribonuclease, deoxyribonuclease, and hot trichloroacetic acid, in that order. The results indicated that during the prepupal period studied: (1) the amount of DNA in puff regions at least quadrupled while that in non-puff regions doubled; (2) the DNA's deposited in the two puff regions studied may differ with respect to the ease with which they are extracted by the reagents used; (3) the protein (residue after extraction with hot TCA) at least doubled in the puff regions but increased by no more than 150% in non-puff regions; and, finally, (4) that RNAase did not extract measurable amounts of material (RNA) from either puff or non-puff regions in these fixed (45% acetic acid) chromosomes. It is concluded that the DNA content of a chromosome strand in a differentiated, somatic cell is not necessarily constant throughout development, but that changes may take place in active regions. It is not excluded that similar changes may occur in other instances in which no disproportionate change could be established within the variation of the data, e.g., in puffs in *Drosophila*.

RUDORF, WILHELM. Genetics of *Phaseolus aborigineus* Burkart. *Max-Planck-Institut für Züchtungsforschung, Köln-Vogelsang, Germany.*

Phaseolus aborigineus Burkart (Burkart and Brücher, Der Zuchter *23*:65–72), a line from Tucuman (our thanks go to Dr. Brücher for providing the seeds) which excels in resistance to the four known races of *Colletotrichum lindemuthianum*, is being used for breeding purposes. Genetics of the pronounced short-day reaction (*neu*+) of the wild bean has been investigated, and it has been found, in short-day series, that *in*+, the factor for indeterminate growth, segregates in a 3 : 1 relation without any disturbances. At the same time *in*+ causes a clear-cut retardation in flowering, compared with *in*. In long-day series there was obtained a 9 : 3 : 4 relation for *in*+ *neu*+ (indeterminate short-day), *in*+ *neu* (indeterminate day-neutral) and [3 *in neu*+ + 1 *in neu*] (determinate day-neutral). Control in F_3 proved that *in* is epistatic to *neu*+.

If we consider as susceptible only plants which after infection with the four race groups of *Colletotrichum lindemuthianum* die like the susceptible parent and as resistant all those plants which either show no sign of infection (like the wild bean) or recover completely, then the genetics of resistance is: race groups α, γ, δ, 9 resistant : 7 susceptible; race group β, 3 resistant : 1 susceptible. Observations on segregation in quantitative characters, such as pod length and broadness as well as weight and size of seed, proved that the genetics of these characters follows the same line as in crosses of races or varieties of the species *Phaseolus vulgaris*.

In crosses with varieties of *Ph. vulgaris* fertility is complete and segregation quite normal. The genomes of *Ph. vulgaris* and *Ph. aborigineus* therefore seem to be homologous, and varieties of the cultivated garden-bean will have developed from the wild bean by different mutation steps.

RUDORF-LAURITZEN, M. The Trisomics of *Antirrhinum majus* L. *Max-Planck-Institut für Züchtungsforschung, Köln-Vogelsang, Germany.*

From crosses of tetraploid with diploid forms of *Antirrhinum majus* L. ($2n = 16$) $3n$ and $3n \pm 1$ or 2 plants have been obtained, which again were crossed with diploids.

Whereas $3n$ plants gave 114 good seeds out of 144 crosses, $3n \pm 1$ or 2 gave only 2 seeds of 68 crosses. The descendants consisted of 44.44% diploids, 42.86% trisomics, the rest being $2n + 1$ fragment, trisomics + 1 fragment, tetrasomics, tetrasomics + 1 fragment, pentasomics and triploids. The trisomics could be distinguished by their phenotype, which differed from normal diploids as well as from each other in many characteristics. Out of the possible 8 trisomics 6 had in 1932–3 been found spontaneously by Stubbe: Anaemica, Purpurea, Rotunda, Contracta, Candida and Fusca. New in appearance are the last two, Plicata and Lucens. The fertility of the trisomics, as determined by the amount of functional pollen, varies from 0% to about 60%. The difference can be high even between single flowers in the inflorescence of one plant. As concerns the number of germinating seeds, when the trisomics are used as mother, a more constant value is obtained. The descendants of trisomics × diploids give from 5–20% trisomics depending upon the particular trisomic concerned, the rest being diploids and aneuploids other than trisomics. If trisomics are used as father, only diploids are recovered.

The trisomics have been crossed with marker genes of the 8 existing linkage groups. From the segregation results after backcrossing until now 6 of the linkage groups could be associated with particular trisomics.

Cytological studies of these chromosomes will make it possible to co-ordinate the linkage groups with the morphologically destined chromosomes.

Russell, Elizabeth S., Seldon E. Bernstein, and Lois J. Smith. Permanent Implantation and Continuing Function of Normal Blood-Forming Tissue in Genetically Anemic Hosts. *Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.*

Any double-dose combination of the deleterious genes of the *W*-series in the mouse (*W*, W^v, W^j) causes severe macrocytic anemia. Animals of the most severely affected genotype, *WW*, die shortly after birth. Haematopoietic cells from livers of 15 half-day normal embryos can become established in histocompatible anemic *WW* or WW^v hosts, and will continue to function autonomously according to their *ww* genotype. The hosts acquire a blood-picture indistinguishable from that of normal *ww* animals. The long-term effects of implantation of 8×10^6 isologous embryo-liver cells, following 200 r X-irradiation of the host, have been followed for 7 WW^v anemics and 10 normal littermates, with determinations of erythrocyte number and size made at 50-day intervals. From 510 to 720 days after treatment, the blood-picture of the anemics remains similar to that of *ww* animals of the same age. Attempts at implantation of homologous embryo liver cells in radiated anemic hosts have been unsuccessful with radiation doses of 200 r, 800 r, and 950 r. Prior radiation of hosts is not necessary for implantation if host and graft are completely histocompatible. Successful implantation of isologous *ww* embryonic haematopoietic tissue without radiation of the host has been attained as follows: 4 of 5 adult WW^v hosts; 9 of 14 juvenile (6–25 days old) WW^v hosts; 4 of 15 juvenile (7–13 days old) mice of the lethal *WW* genotype. The criterion of success was attainment and maintenance of erythrocyte number and size characteristic of normal *ww* mice. The erythrocyte level of implanted non-radiated anemics occasionally fluctuated for some time before becoming established at a permanent high level. Isologous *ww* cells implanted in non-radiated *WW* and WW^v animals compete successfully with the

less active cells of the host and eventually completely dominate the blood-picture of the host.

RUSSELL, LIANE BRAUCH, and W. L. RUSSELL. Genetic Effects of Radiation in the Female Mouse. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

All stages of gametogenesis occur in the adult male mouse. In contrast, in the adult female, oogonia are not present, only the range from primary to secondary oocyte is represented, and, since meiotic metaphase I occurs only shortly before ovulation, the bulk of germ cells are primary oocytes, probably in dictyate stage. Studies of genetic effects of radiation in females therefore assume a unique position in that they deal exclusively with a gametogenic period whose products are not separately recoverable in the male. Furthermore, the dictyate state has not been observed in spermatocytes.

The major difficulties that had heretofore interfered with work on females—X-ray sterilization, and the danger of confusing genetic with maternal effects—have been analysed and resolved.

The living embryo/corpus luteum ratio accurately measures dominant lethal induction in females while litter size does not. The X-ray induction of 50% dominant lethals requires about 700 r in primary oocyte stages, but only 70 r at or near first meiotic metaphase. Proof of dominant lethality was established in both cases by demonstrations that death occurs after fertilization (most of it after 2-cell stage) and that about 60% of all eventual deaths are already represented by gross nuclear aberrations after the first cleavage.

The translocation frequency recovered from oocytes irradiated 1½–34½ days before ovulation is considerably lower than that recovered from irradiated sperm. Incomplete results from a pilot study on deleterious first generation effects indicate a shortening of life span in sons of irradiated females.

The specific locus mutation rate in oocytes exposed to 300 r continuous gamma-radiation at about 100 r per week is considerably lower than the rate in spermatogonia given 300 r acute X-rays.

RUSSELL, W. L., JEAN W. BANGHAM, and JOSEPHINE S. GOWER. Comparison between Mutations Induced in Spermatogonial and Postspermatogonial Stages in the Mouse. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

Male mice exposed to 300 r of X-rays were each mated to two females a week for six weeks following irradiation. The 33,874 offspring obtained were observed for mutations at seven specific loci. The mean mutation rate per locus obtained in this particular sampling of postspermatogonial stages is twice the rate found in earlier experiments with spermatogonia. However, the difference is not merely quantitative: the nature of the mutations recovered in the two experiments is quite different.

First, and most striking, in the experiments with spermatogonial stages no deficiencies involving both of the closely linked *d* and *se* loci (c.o. 0.16%) were found, although 22 induced mutations at the *d* locus and 3 at the *se* locus were observed. In contrast, the experiment with postspermatogonial stages, which, being less ex-

tensive, has yielded only 3 mutations at the *d* locus and 2 at the *se* locus, has, nevertheless, also given 5 mutations involving both *d* and *se* loci. Since at least 3 of these are transmitted to descendants, loss during gametogenesis does not appear to be the explanation for the absence of this type of deficiency in the mutations recovered from irradiated spermatogonia. Apparently these deficiencies are not commonly induced in spermatogonia.

Another contrast is the smaller variation in mutation rates among the seven loci in the experiment with postspermatogonial stages, there being only about a threefold difference between the lowest rate and the highest, whereas in the spermatogonial experiments the difference is more than thirtyfold. When this information is combined with the above, it appears that mutations recovered from irradiated spermatogonia are mostly point mutations with wide differences in mutation rate, whereas those recovered from irradiated postspermatogonial stages contain a large contribution from deficiencies and probably other aberrations which tend to equalize the rate at all loci.

RUSSELL, W. L. *See* RUSSELL, LIANE BRAUCH.

RYAN, FRANCIS J. Mutation as an Error in Gene Duplication. *Department of Zoology, Columbia University, New York, N.Y., U.S.A.*

In stationary phase populations of *Escherichia coli* mutants have been observed to appear. These have been shown to be due to genotypic changes occurring during that period. The possibility that these mutations occur during the cryptic growth of cells involved in a population turnover has been eliminated by several experiments including some utilizing penicillin and others depending on the development of adaptive enzymes.

Further investigations have been made on the question whether in these non-dividing bacteria there is the continued synthesis of polymers that might be concerned with gene mutation. Since direct chemical measurements might be difficult to interpret, an indirect approach involving attempts to prevent polymer synthesis was undertaken. Inhibitors and the starvation of double mutants were the means employed for this purpose.

Drugs such as chloramphenicol and growth factor analogues were presented to the non-dividing bacteria and it was determined whether the stationary phase mutations occurred in their presence. On the other hand, doubly marked strains, requiring amino acids, vitamins or nitrogen bases as well as being mutant at the locus under investigation, were starved for the substances mentioned. The rate of occurrence of stationary phase mutations under such conditions was compared with that occurring in properly supplemented cultures. Although in all cases the effect of these treatments on the synthesis of protein, RNA and DNA could only be inferred, the pattern with which the treatments prevented the occurrence of mutations allows the conclusion that these mutations require polymer synthesis and provides a clue as to what compounds are involved. The results are consistent with the hypothesis that gene duplication is necessary for most if not all of even these rare mutations which occur in the absence of cell division.

SACHER, GEORGE A. *See* GRAHN, DOUGLAS.

SACHS, LEO. The Possibility of Directed Immunogenetic Changes in the Cells of Mammalian Tumors. *Weizmann Institute of Science, Rehovoth, Israel.*

There are some malignant tumors that are unlike normal tissues in that they can give successful homotransplants: i.e., they grow progressively when grafted into animals with different genetic constitutions. The immunogenetic properties of such tumors in mice have been analysed by studies on: (1) the antigenic composition and antigenicity of the agglutinogens; (2) the types of antigens that elicit cytotoxins detectable by the addition of complement; (3) the degree of cytotoxic immunity evoked by tumor homografts; and (4) the chromosomes of the tumor cells. These studies have shown that although homotransplantable tumors may or may not have lost certain agglutinogens and cytotoxinogens detectable by the addition of complement, all those investigated have gained antigenicity. The ability to grow progressively in a foreign host is thus due not to the loss of antigenicity, but to a gain of resistance by the tumor cells to the iso-immune response. This gain of resistance seems to be due to an extra- or intracellular neutralisation by antigens of the tumor cells of the cytotoxic factors produced by the foreign host.

In experiments by others on the induction of immunological tolerance to tumor cells, it was shown that under certain conditions tumors can acquire the property of homotransplantability. The ability of these tumors to give successful homotransplants is also due to a gain of resistance to the immune response. There is evidence that this resistance may be acquired by cells directly as a result of growth under conditions of a weak immune response such as exist in partially tolerant animals. This therefore suggests the possibility of obtaining directed genetic changes in mammalian cells.

SAEZ, FRANCISCO ALBERTO. Chromosome Evolution in *Dichroplus*. *Instituto de Investigacion de Ciencias Biológicas, Montevideo, Uruguay.*

This paper is a general survey of the variation and evolution of chromosomes in some species of the genus *Dichroplus* (Orthoptera: Acrididae) found in Uruguay. A numerical series ranges from the supposed ancestral karyotype, $2n$ (δ)=23, to the extraordinary unexpected number of $2n$ (δ) = 8. *Dichroplus punctulatus*, *D. conspersus*, and *D. elongatus* have 11 pairs of acrocentric chromosomes and the sex chromosomes free. Variation was found in *D. bergi* with $2n$ (δ) = 22, 10 pairs of acrocentrics and 1 metacentric chromosome formed by fusion of the sex chromosome with an autosome (neo-Y, neo-X system) while the number of major chromosome arms is not reduced, remaining 23. *D. pratensis* (Bruner) has $2n$ (δ) = 18, 8 pairs of acrocentrics, 1 metacentric autosome and the sex chromosome free, but the number of chromosome arms is reduced to 19. Another form of *D. pratensis*? has $2n$ (δ) = 18 with 6 pairs of acrocentrics, 2 metacentric autosomes and 1 trivalent formed by fusion of the sex chromosome with a bivalent autosome (neo-Y, neo-X system), the number of chromosome arms remaining 23.

In Acridians, *D. silveira guidoi* (Liebermann), with $2n$ (δ) = 8, is extremely strange and unique: the karyotype is composed of 2 pairs of metacentric autosomes, 1 pair of acrocentric autosomes and a heterozygous acrocentric pair which is believed to represent the X-Y sex chromosomes. The whole number of chromosome arms is 12, meaning that 11 chromosomes had been "lost" of the original diploid set.

The above-mentioned species were found over all the Uruguayan territory except *D. silveira guidoi*, whose dispersion area was restricted to a small region in the

northern part of the country. The striking mutation found in *D. Silveira guidoi* has been established in nature, having singular adaptive value, and is maintained in the population with few changes in the viability of the individuals. The probable mechanisms involved in the production of such exceptional evolutionary changes are discussed.

SAGER, RUTH. Non-chromosomal Inheritance in *Chlamydomonas. Department of Zoology, Columbia University, New York, N.Y., U.S.A.*

The discovery of a non-chromosomal determinant of streptomycin resistance in the sexual alga *Chlamydomonas reinhardi* was reported at the Genetics Congress in 1953; its uniparental transmission in crosses and its phenotypic stability were described. Subsequent studies of this material have centered on the question of the nature of this determinant and of the mutational process underlying its alteration. Evidence will be presented concerning the conditions under which mutated strains are recovered, and the implications of these findings will be discussed.

SAKAMOTO, S. *See* MATSUMURA, S., *et al.*

SAMPSON, D. R. Inheritance of Shades of Bronze and Pink Flower Colors in *Antirrhinum majus. Horticulture Division, Central Experimental Farm, Ottawa, Canada.*

Interaction effects of the genes *Inc*, *Pal* and *Rd* on intensity of anthocyanin pigmentation in flowers of *Antirrhinum majus* were studied in *eos eos* material. All possible combinations of the three genes were obtained in both *sulf sulf* (bronze) and *Sulf* (pink) plants. Flowers of *inc inc* or pal^{rec} pal^{rec} plants are acyanic. Both *Inc* and *Pal* are incompletely dominant and to the same extent. *Rd* is a fully dominant anthocyanin intensifier. *Inc inc*, *Pal* pal^{rec} and *rd rd* genotypes weaken the intensity of pigmentation equally. Interactions of the three genes result in four shades of both bronze and pink flowers.

SÁNCHEZ-MONGE, ENRIQUE. Rye Genome in "Terminillo" Wheat. *Centro de Mejora del Maiz del Instituto Nacional de Investigaciones Agronómicas, Madrid, Spain.*

The "Terminillo" wheat was obtained by the late Italian breeder Nazareno Strampelli from the back-cross (Rieti wheat $\times$ rye) $\times$ Rieti. Both forms, Terminillo and Rieti, can be classified botanically as *Triticum aestivum* L. var *erythrospermum* (Körn.) MSF., but they present some marked morphological differences. It can be reasonably assumed that such differences are due to the introgression of rye genes

into the Rieti wheat. However, the possibility of such introgression is denied by several authors. Morphological and cytological proofs will be given to demonstrate that such introgression does occur.

SANDERS, MARY E. *See* ROSS, JAMES G., *et al.*

SANG, JAMES HENDERSON. Selection for Larval Development Rate Using Aseptically Cultured *Drosophila*. *Agricultural Research Council Poultry Research Centre, Edinburgh, Scotland.*

Larval development rate in *Drosophila* has a heritability of 20–25% and the character shows a notable degree of hybrid vigour in crosses. Selection from a cross shows progress only in the direction of slower development (Sang and Clayton, J. Hered. *48*:265) apparently since the fast developing larvae are always heterozygotes. As this situation may be typical of some cases of asymmetrical response to two-way selection it is important to explore the physiological mechanisms which may be involved. Earlier work (Sang, Caryologia, vol. suppl. 818) suggested two possibilities, both related to protein metabolism, which could be examined using aseptic nutritional techniques. Selection for larval development rate was therefore carried out on a diet deficient in casein and on a diet deficient in pyridoxine, which is the main vitamin involved in amino acid metabolism. In the first case, selection was successful only in the direction of slow development whereas responses were obtained initially in both directions using the pyridoxine deficient medium. Later the response declined in this case also, the heritability becoming zero.

Examination of the changes of some nutritional requirements resulting from both courses of selection will be described, and compared with what is known of the nutritional needs of pure lines and crosses. The limitations of nutritional techniques for this type of physiological study will also be considered.

SANTIBANEZ, S. KOREF. *See* PASQUALE, A. DI.

SARVELLA, PATRICIA. *See* BLICKENSTAFF, JOYCE; LIMA-DA-FARIA, A., *et al.*

SATO, IWANE, and IGOR L. KOSIN. Chromosome Complements in Parthenogenetic Turkey Embryos. *Department of Poultry Science, State College of Washington, Pullman, Wash., U.S.A.*

Work at the State College of Washington has demonstrated that turkey eggs from virgin hens show relatively high incidence of parthenogenetic development (Proc. Soc. Exper. Biol. Med. *93*:605). However, in only 4% of germ discs does truly embryonic formation occur; in 30% of the discs the development is limited to mem-

brane and blood island formation. The development of such parthenogenetic embryos is retarded. For example, following 7 days of incubation the average parthenogenetic embryo approximates the stage of development reached by "normal" embryo after only 3 to 4 days of incubation. This delay seems to be due to low mitotic index of parthenogenetic embryos.

The diploid chromosome complement of turkeys is 18 macrochromosomes (including 2 sex chromosomes) for the male, and 17 (including 1 sex chromosome) for the female, and about 60 microchromosomes for both sexes. Chromosome counts on embryos and undifferentiated tissue from virgin eggs show nearly all nuclear plates of mitotic metaphase to be of diploid configuration. This suggests early duplication in parthenogenetically developing tissues. Our observations indicate the existence of considerable variation in the chromosome number of somatic diploid cells from parthenogenetic embryos and tissues. In these there also exist small nuclear plates with low chromosome numbers of the order of 10 macrochromosomes and 30 microchromosomes. Such haploid-like configurations can be seen not only in the undifferentiated cell groups but also in certain types of embryonic tissue cells, especially in erythroblasts, fibroblasts and epithelial cells. In these specimens the recovery of diploidy must have occurred after at least one division of the haploid female pronucleus: some cells then remained haploid without duplication. Doubling of chromosome sets frequently occurred in the diploid cells of parthenogenetic germ discs, leading to tetraploidy.

SAUL, GEORGE B. *See* WHITING, P. W.

SAVITSKY, HELEN. Chromosome Behavior in Interspecific Hybrids between *Beta vulgaris* L. and Species of the Section *Patellares* Tr. *Beet Sugar Development Foundation and U.S. Department of Agriculture, Salt Lake City, Utah, U.S.A.*

Hybrids between *Beta vulgaris* and species of the section *Patellares* are usually lethal and die in the seedling stage. Rare hybrids from Swiss chard $\times$ *B. webbiana* (obtained by J. O. Gaskill) survived but were completely sterile. The first semi-fertile hybrid was obtained by Dr. R. K. Oldemeyer of the Great Western Sugar Company from Turkish leaf beet $\times$ *B. procumbens*. Meiosis was studied in Gaskill's sterile hybrids, Swiss chard (*B. vulgaris*) $2n \times$ *B. webbiana* $2n$ and in Oldemeyer's semi-fertile hybrids Turkish leaf beet (*B. vulgaris*) $2n \times$ *B. procumbens* $2n$. Degrees of chromosome pairing and chiasma frequencies were a little higher in the semi-fertile hybrid with *B. procumbens* but were not sufficient for fertility. A certain grade of fertility in the hybrid with *B. procumbens* was due to the division of univalents and bivalents and to the regular and equal distribution of chromosomes in the first meiotic division. Because of the regularity of the first division, the composition of the chromosome set in some gametes approached the composition of the chromosomes in the non-reduced gametes. Such gametes contained almost a complete set of chromosomes from both species. The haploid gametes containing a few chromosomes from one species and a few from another species were not viable. Only diploid gametes or the gametes with an approach to the diploid number of chromosomes, and which had more chance to contain a complete haploid set of chromosomes of one species, were viable. Interspecific hybrids between two diploid *Beta* species could not survive on the diploid base. The progeny obtained consisted

of triploids and aneuploids approaching to triploids. Different abnormalities in meiotic division were observed: breakage of chromosomes, bridges, telocentric and acentric fragments, non-disjunction, multipolar spindles, fused spindles, etc.

SAVITSKY, V. F. Differences in Interaction of Cytoplasm-Genes in Different Male-Sterile Races in *Beta vulgaris* L. *Beet Sugar Development Foundation and U.S. Department of Agriculture, Salt Lake City, Utah, U.S.A.*

In the process of repeated backcrossing of different *B. vulgaris* populations consisting of hermaphrodite plants, it was established that many hybrid populations segregated for male-sterile plants after backcrossing to monogerm beets. All occurrences of male-sterility were due to the interaction of male-sterile cytoplasm and the nuclear factors which do not restore pollen fertility in the presence of male-sterile cytoplasm. Genetic analysis of hybrids indicated that the appearance of male-sterile plants in backcross hybrids was caused by the presence of male-sterile cytoplasmic races in the multigerm hermaphrodite populations and elimination of the existing system of dominant genes which restored pollen fertility in these male-sterile cytoplasmic races. The process of substitution of dominant genes for recovery of pollen fertility by the genes causing abortion of pollen grains occurs faster in monogerm plants, because of linkage between non-recovery genes and the gene *m* (monogerm character). It is demonstrated that male-sterile cytoplasm isolated from different populations differs in this reaction to nuclear genes which do not restore male-fertility. These differences between plasms in the manifestation of the grade of male-sterility and in the interaction plasma-genes did not change during eight backcrosses and eight generations of selection for male-sterility within male-sterile lines. Different expressions of male-sterility in *B. vulgaris* L. are caused by different genes which influence the cytoplasm, as well as by the different types of male-sterile cytoplasm.

SAWIN, P. B., and D. D. CRARY. Chondrodystrophy (Da) in the Domestic Rabbit. *Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.*

A viable disproportionate dwarfism in the rabbit induced by the *Da* gene (incompletely dominant) results in *generalized* reduction in body size with greater effects specifically *localized*. In the skull, greatest retardation occurs in the endochondral bones. Prenatally, it is focused posteriorly in the basioccipital, but in adults it shifts anteriorly to the sphenoid elements, and corresponding compensatory adjustments occur in the adjacent membrane bones. Basally in the ear is a small papilla identifying homozygotes of all ages, even as early as 16 days prenatally when other characteristics are more subtly expressed. In the limbs the major reductions are proximally in the scapula and ilium, with lesser effects on the length of the more distal units—radius, ulna, metacarpals and metatarsals. Deficient development of glenoid fossa and acetabulum, accompanied by complete absence of the round ligament, may lead to severe adolescent crippling. If not severely crippled DaDa's will mate, conceive, and bear young, but are clumsy and rear their young with difficulty. In the vertebrae (and to a lesser degree in limbs) the generalized reduction can be recognized in the fetus at least as early as 22 days, and persists through life. Secondarily, it *interacts* with the species pattern of localized retardation of growth at successive ages (observations taken on 21 and 22 days prenatally, at birth, and in the adult)

reaching its greatest expression in length or width of those parts or regions which are growing most actively at the time. These then are temporarily the critical regions. They are not the same through life, but change progressively with age, apparently an adjustment of regional gradients to generalized retardation. Histologically, the proliferative zone of cartilage shows an increase in intercellular matrix and consequent disorganization of the columns of hypertrophied cells, the relative effects of which appear to be similarly localized in time as well as place.

SCHACHT, LEE E. *See* GERSHOWITZ, HENRY.

SCHALET, ABRAHAM. A Study of Spontaneous Visible Mutations in *Drosophila melanogaster. Department of Zoology, Indiana University, Bloomington, Ind., U.S.A.*

Over 490,000 daughters of *Drosophila melanogaster* obtained by Muller's "Maxy" technique were examined for spontaneous visible mutations arising at 13 loci in their paternally derived X-chromosome. Simultaneously over 340,000 sons were examined for mutations in their maternally derived X-chromosome. Since sons were not scorable for lethal alleles or isoalleles, poorly scorable for mutations of wing and eye conformation, and often failed to breed, the estimates of mutation frequency given below are based on the daughters. At least one transmissible mutation involving each locus was found. The per-locus frequency of transmissible *independent* mutational events (totalling 39) averaged $(6 \pm 1) \times 10^{-6}$, and ranged from 9 for cut, down to one each among 490,000 for prune, echinus, outstretched small eye, carnation. The frequency of genetically proved *mutants*, including all those of common origin (a cluster of 10 cuts, one of 2 cuts, and one of 3 yellows) equally with the 36 that arose singly, averaged $(8 \pm 2) \times 10^{-6}$ per locus. Tests of lethal frequency among representative chromosomes of paternal origin gave 32/8,706, about double the rate sometimes considered as standard. This would make the "standard" per-locus rates of visible mutations about half of those above. Since several cases were proved deficiencies, the per-locus rates for gene mutations are somewhat lower still. The ratio of fractional to apparently whole-body *independent* mutations was 20:19 for proved alleles of specific loci and 38:21 for untransmitted "mutations" (including very few possible phenocopies) of fertile individuals having corresponding phenotypes. Since even the last cited 21, being untransmitted, must have been fractional, there are only 20 possibly whole-body to nearly 78 fractional cases. Most spontaneous mutations therefore occur in early cleavage and/or mature germ cells. Moreover, for every individual with a newly arisen germinal mutation there are some 3 or 4 that undergo comparable impairment owing to somatic mutation. (This work was supported by a grant to Dr. H. J. Muller and associates from the U.S. Atomic Energy Commission (Contract AT (11–1)–195).)

SCHECROUN, JANINE. Sur la réversion provoquée d'une modification cytoplasmique chez *Podospora anserina. Faculté des Sciences de Paris, Laboratoire de Génétique, Paris, France.*

Chez l'ascomycète *Podospora anserina*, le couple de gènes *S s* conditionne la formation d'un barrage entre souche *S* et souche *s*. Ce barrage n'empêche pas la fertilité

du croisement $S \times s$, et dans sa descendance, on trouve régulièrement à la place des souches *s* des souches modifiées s^S qui ne donnent pas de barrage avec S. La forme s^S est interprétée comme résultant d'une modification cytoplasmique de la forme s. s^S peut réverser vers *s* spontanément avec fréquence faible, ou régulièrement par suite d'un contact cytoplasmique entre filament s^S et filament *s* et en l'absence de toute migration de noyaux s. Lorsque la réversion a débuté en un point, elle progresse à l'intérieur du filament d'une manière continue; la vitesse de progression croit beaucoup avec la température et peut atteindre 7cm./jour dans les meilleures conditions.

Si l'on admet que : *s* et s^S ont le même noyau, que *s* possède dans son cytoplasme des éléments particulaires, que ces éléments absents dans s^S peuvent y apparaître spontanément et s'y multiplier, que *S* inhibe leur multiplication, on peut rendre compte de tous les faits connus.

SCHERR, GEORGE H. *See* RIPPON, JOHN W.

SCHMALHAUSEN, IVAN. Hereditary Information and Its Transformations. *Academy of Sciences of the USSR, Moscow, U.S.S.R.*

The mechanism of evolution is regarded from the viewpoint of cybernetics. Since population is the least evolving unit, it is the regulated object. Biogeocoenose plays the role of the regulator. Population is connected with it by two channels of communication. The first is on the molecular level of organization and transmits hereditary information from the zygote to primitive germ cells of the adult individual. The second channel, on the organization level of an individual, transmits feed-back information from phenotypes to the biogeocoenose. Between these two channels transformation mechanisms connect the channels and circuit the elementary cycle of evolutionary changes. Alternation of two pairs of bases in the molecule of DNA gives a vast number of possible combinations ensuring deep individuality of each molecule, since the number of the links in a long chain molecule is great. Hereditary information is transmitted through the mechanism of cell-division by chromosomes which are the regulators of intracellular metabolism and individual development. The essence of individual development, realized in morphogenous mechanisms by the use of amplifiers, consists in transformation of hereditary information into a system of life relations of the phenotype to its environment. Natural selection is accomplished on the basis of the evaluation of the phenotype. This is the mechanism of transformation of the feed-back information in the biogeocoenose. Transformation is completed by the reorganization of hereditary information in zygotes. Random environmental influences are "noises" disturbing the information. Elimination of deviations, i.e., stabilizing selection, leads to the increase of "noise" reduction. Reiteration of information is realized by repetition of similar genes, of their complexes and of entire chromosomes in a diploid organism. The system of internal connections is used both in the structure of genes and chromosome linkage and in the set arrangement of chromosomes. The hereditary code is isolated by its position under the protection of regulatory mechanisms of the cell. Stability of the transforming mechanism of information in ontogenesis is maintained by means of internal relations in the regulatory systems. Reliability of the feed-back information is ensured by the organization of the phenotype, by repetition of all essential characters in each individual, by means of protection and isolation from disturbances.

Scholz, F. Smooth-awned Barley Mutants Induced by X-Rays. *Institute for Research of Cultivated Plants, Gatersleben, of the German Academy of Sciences, Berlin, Gatersleben, Kreis Aschersleben, Germany.*

Mutation experiments by means of X-rays have been pursued at Gatersleben (Germany) for about twelve years on both spring and winter barley. Up to now about 800 mutants have been isolated. Among many high-yielding mutant strains which are valuable for breeding purposes, there are several semismooth mutants, such as a smooth-awned one (Mut. 4033), of special interest. Most of the mutants descend from the two-rowed, rough-awned variety, Haisa. In yield trials since 1951 these mutants have been unquestionably superior to Haisa. But another smooth-awned mutant (Mut. 4034) has shown high-grade partial sterility (on an average 70–80%).

Several authors emphasize that smoothness of awns is very closely associated with hairless stigmas. This property may prevent the normal reception of the pollen, resulting in a relatively high number of sterile spikelets, and eventually in lower yields. Our high-yielding Mut. 4033, however, has moderately haired stigmas; its fertility is nearly normal. Moreover we can show that smooth-awned barleys in the Gatersleben World Collection may have all gradations from hairless to normally haired stigmas.

Crossing the mutants with each other as well as with Haisa indicated three independent recessive genes for semi-smoothness. In both the smooth-awned mutants a multiple allelic series is involved which is independent of the above-mentioned genes. The dominant allele is responsible for rough awns of Haisa. Smoothness of the awns of Mut. 4033 is governed by the one recessive allele. The third allele (Mut. 4034), being recessive to that of Mut. 4033 also, is responsible for the same grade of smoothness as well as for extreme partial sterility and, to all appearances, hairless stigmas. Thus in one locus there may be various mutations for smooth awns affecting hairiness of stigmas and fertility differently. Studies on allelic relations to r-genes have been started.

Schreiber, Giorgio, A. R. Hoge, H. W. Belluomini, and A. M. Penha. Further Researches on Intersexuality in a Highly Isolated Population of Snakes. *Instituto de Biologia, University of Minas Gerais and Instituto Butantum, Sao Paulo, Brazil.*

At the IX International Congress of Genetics (1953) we presented the problem of *Bothrops insularis* Amaral, a crotalid from the island of Queimada Grande (South Atlantic–Brazil). Further results of 5 years of research are now reported, with new statistical data and embryological findings.

This snake presents the sexual abnormality of the presence of a hemipenis (bilateral or unilateral) in a great majority of the females. The true females (without hemipenis) are probably sterile and the females with hemipenes bear a smaller number of developed embryos than do females of related species.

Three problems have been studied: first, the genetic sex of the abnormal individuals; second, the development of the gonads in normal and abnormal embryos; and third, the sex ratio in the population during almost 35 years of capture.

The snake is restricted to this island and no other crotalid snake exists in the same territory (almost half a square mile of surface).

The embryological researches show that those with testis always bear well-developed bilateral hemipenes. The embryos with ovaries and Mueller ducts can

bear bilateral hemipenes (more or less developed), unilateral hemipenes (generally right) or none (true females). The abnormal individuals are thus embryologically females.

The genetical sex of the abnormal individuals has been determined by the statistical analysis of the scaling. The ventral scale number is a true somatic sexual character being determined very early during the segmentation of the mesoderm into somites and thus independent of the influence of gonadic sex hormones.

The statistical distribution of this character in the abnormal individuals shows clearly that it falls in the field of the true females. A further statistical analysis carried on with the method of the "discriminant function" between two groups of three characters each confirms this result with considerable evidence.

The sex "ratio" in the population shows a trend of reduction of number of males and true females, and increase of the number of abnormals. The fact that the abnormal individuals are genetically females indicates that their increase is independent of the decrease in the number of males probably carrying a lethal factor and is not due to a transformation of the male gonads into ovaries.

The significance of the abnormality is considered and the term *intersexuality* (Padoa) is used here in a broad sense. A new term "arrenoidism" (females bearing a unique male character) is suggested and the problem of the probable extinction of the species in the island because of the continual and evident diminution of the males is discussed.

SCHULTZ, JACK. The Nature of Variegation in *Drosophila*. *Institute for Cancer Research, Philadelphia, Pennsylvania, U.S.A.*

The variety of types of mosaic found in *Drosophila* fall into a number of categories: heterochromatin-induced variegation, that is, changes in the expression of loci correlated with transposition to or from heterochromatic regions; gross chromosome changes; somatic crossing-over; somatic mutation *sensu stricto*, and miscellaneous variations in patterns of mutant expression, correlated with cell lineage. The last group is a nondescript one at present; perhaps it provides material for the analysis of the genetics of differentiation. The others are better understood.

The genetic system underlying heterochromatin-induced variegation may be considered responsible for the integrative control of nucleic acid synthesis in the organism. The lines of evidence supporting such a statement are: (1) the cytological and cytochemical analysis of changes adjoining the loci of the associated rearrangements; and (2) the metabolic analysis thus far of the variegation phenomenon (response to antimetabolites affecting nucleic acid synthesis to changes of nutrition, etc.). Against this background, the genetic inter-relations of the numerous modifiers of variegation will be examined, and their relation to the heterochromatic regions, both intercalary and centric, discussed. The various degrees of specificity displayed by these modifiers, and their relationship to the effect of distance from point of rearrangement and to other properties of the variegation phenomenon, will be shown.

The apparent contrast between the variegation due to somatic crossing-over and the heterochromatin-induced variegation will be examined as an expression of the relation between the behavior of the chromosomes as orgamelles (somatic crossing-over) and the replication of their individual units. The relation between the maize and *Drosophila* variegation will also be considered in these terms. Finally, the inter-relations between these problems and those of nuclear differentiation during development will be discussed.

SCHWARTZ, DREW. A New Mutable Gene in Maize. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

A spontaneously occurring mutational system at the *c* locus in maize is under investigation. This highly mutable recessive allele, c^m, mutates to both a stable *C* and a stable *c*. Mutations can occur in the sporophyte and gametophyte, as well as in the endosperm. The mutability of c^m is autonomously controlled and is not associated with any detectable chromosome breakage. It is completely independent of the *Ac-Ds* system.

The c^m gene is stable in the zygote and does not mutate. This stable condition is maintained until some time during ontogeny when the gene becomes unstable and free to mutate. The time at which the shift to instability occurs is determined by the state of c^m. Three distinct states can be recognized. In the late state, the stability is maintained until after fertilization and mutations occur only in the endosperm. In the intermediate state, the shift occurs some time around meiosis so that some mutations occur before fertilization, giving rise to self-colored kernels. In the early state, the c^m gene becomes unstable in the young sporophyte giving rise to large mutant sectors on the ears. At fertilization, those genes in the zygotes that have not as yet mutated revert to the stable condition and the cycle is repeated.

SCHWEMMLE, JULIUS, C. G. ARNOLD, and H. O. GLENK. Preuve et bases chimiques de la fertilisation sélective chez les Œnothères. *Botanisches Institut der Universität, Erlangen/Bayern, Allemagne.*

La fertilisation sélective *sensu stricto* doit être opposée à ces processus de sélection qui empêchent une combinaison libre et fortuite des gamètes avant que les cavités des ovaires soient atteintes par les tubes polliniques (concurrences des tubes polliniques, du sac embryonaire, facteurs de létalité entre autres).

La fertilisation sélective *s.s.* repose sur une réaction chimique directe entre gamètes mâles et femelles.

C'est Schwemmle à Erlangen qui a prouvé clairement avec des espèces d'œnothères et pour la première fois ce phénomène depuis longtemps discuté. Les espèces d'œnothères sont particulièrement favorables par suite de leur constitution génétique (formation des complexes des chromosomes).

Lors du croisement de *Oenothera Berteriana* (symbole de complexe B et l) avec *O. odorata* (v et I) la combinaison B·v manque. On peut éliminer toutes les causes qui pourraient expliquer le manque de B·v excepté la fertilisation sélective. Une preuve directe par la méthode de pollinisation complémentaire est possible (plantes B·l pollinisées par du pollen v donnent seulement l·v; une pollinisation suivante et complémentaire avec pollen I donne alors toujours B·I). Ainsi les ovules B n'attirent pas les tubes polliniques v.

On a constaté aussi plus tard une fertilisation sélective dans d'autres espèces d'œnothères.

Les ovules secrètent des substances qui attirent chémotropiquement les tubes polliniques. La force de l'attraction est différente suivant la constitution génétique des gamètes: les gamètes ont des affinités différentes les uns par rapport aux autres. Mesure pour l'affinité: taux d'apparition d'une certaine combinaison zygotique de complexes (par exemple, affinité B — v = O). L'affinité entre ovules et tubes polliniques peut être aussi constatée *in vitro* (expérience de chémotropie).

But des recherches suivantes : isolement et identification des substances à action chémotropique secrétées par les ovules = "Gamones" des plantes supérieures.

SCHWINCK, ILSE, and ELIJAH ADAMS. Biosynthesis of Tyrosine from Prephenic Acid in *Escherichia coli* Mutants. *New York University College of Medicine, Department of Pharmacology*, and *Columbia University, Department of Zoology, New York, N.Y., U.S.A.*

Tyrosine is made from phenylalanine in animals, but not in *Escherichia coli* as indicated by growth experiments with mutants. Direct proof of this has been obtained in recent studies (Schwinck and Adams, Fed. Proc. *17*, in press, 1958) of a soluble enzymatic system which catalyzes tyrosine synthesis from a non-aromatic precursor, the cyclohexadien prephenic acid (PPA). PPA had already been established as a phenylalanine precursor in *E. coli* (Weiss *et al.*, Science *119*: 774, 1954). An independent aromatization in tyrosine formation leads from PPA to p-hydroxyphenylpyruvic acid (HPP), which is convertible to tyrosine by transamination. Prephenic dehydrogenase, the enzyme catalyzing the reaction to the phenolic compound, was partly purified and found to require DPN. Quantitative assays and identification were based on UV-spectra, derivatives and paper chromatography. Essential support for the independence of the aromatization reactions to phenylalanine or to tyrosine comes from enzyme studies with mutants: prephenic dehydrogenase is absent from two independently derived tyrosine auxotrophs which do not require phenylalanine for growth, but the enzyme is active in extracts of the phenylalanine auxotroph 83-5. Mixed incubations with extracts from separate mutants revealed no inhibitory effects. An observation of separate interest indicated that addition of tyrosine to the growth medium of 83-5 results in markedly depressed specific activity of the prephenic dehydrogenase, suggesting this system as an additional case of negative feed back control of the enzyme.

SCOSSIROLI, RENZO EDOARDO, and SAVINA SCOSSIROLI. Response to Selection for Quantitative Traits in Isogenic Populations of *Drosophila melanogaster*. *Istituto di Genetica, Pavia, Italy.*

Selection for sternopleural hairs was started in two isogenic populations of *Drosophila melanogaster* soon after isogenicity was reached. Both control and X-rayed lines responded promptly to selection. The results are interpreted in terms of mutations in the polygenic systems responsible for bristle number. Lines derived from reciprocal crosses between isogenic strains also responded to selection. In this case recombination within polygenic systems probably plays the major role in determining the response to selection.

SCOSSIROLI, SAVINA. *See* SCOSSIROLI, RENZO EDOARDO.

SCOTT, J. P., and JOHN L. FULLER. Genetics and Social Behavior in Dogs. *Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.*

This paper reports results from a ten-year experiment whose purpose was to discover the extent to which heredity can affect behavior (and particularly social behavior) within a species, and the mechanisms through which this is accomplished.

Dogs of five different breeds representing the important groups selected by

breeders for behavioral differences were raised in a standard environment and given a series of behavioral tests and measurements from birth until one year of age. Two breeds, African basenjis and American cocker spaniels, were used in a Mendelian cross, and the hybrids studied in a similar manner. The data from some 450 animals were eventually studied by analysis of variance, factorial analysis, and other statistical techniques.

Breed differences were important in most of the variables studied, but individual (including environmental) and substrain variation was always present in considerable amounts.

The hybrid populations showed several different patterns of inheritance in different behavioral variables: dominance, no dominance, hybrid superiority, and complex interaction. Differences between reciprocal crosses were important in several cases. Multiple factor inheritance was indicated in nearly all.

Breed differences can appear at any age, and may either be magnified or reduced by a constant environment. Variability of behavior in an individual is decreased by habit formation. Each breed inherits a number of basic capacities and simple behavior tendencies which can be easily organized by learning into the complex capacities for which the breed has been selected. Behavior is therefore in part genetically organized through simple behavior patterns and gene combinations, and in part environmentally organized through learning and habit formation. Possible applications to human problems are discussed.

SCOTT, J. P. *See* FULLER, J. L.

SEARS, E. R., and M. OKAMOTO. Intergenomic Chromosome Relationships in Hexaploid Wheat. *U. S. Department of Agriculture* and *University of Missouri, Columbia, Mo., U.S.A.*

The 21 chromosomes of *Triticum aestivum* can be placed in 7 homoeologous groups of 3 according to the ability of tetrasomes to compensate for particular nullisomes. Within these groups all the 42 possible tetrasomic-nullisomic combinations are nearer normal than the nullisomics themselves. Between the groups nearly 50 different combinations have been tested, without any of them showing compensation. The high degree of compensation exhibited in most of the combinations within groups indicates that almost every chromosome is very similar to its two homoeologues in gene content. The homoeologous chromosomes show little tendency to pair, however, for the bivalent frequency in haploids normally averages only about one.

That the pairing in haploids usually involves homoeologous chromosomes has been shown by analysis of translocations recovered from haploids, where each translocation is presumably the result of haploid pairing and crossing over. Of the 13 translocations analysed, 9 involved homoeologous chromosomes: VI–XIX (4 occurrences), II–XX (2), XI–XXI (2), and XII–XVI. The other 4 were: II–VIII, IV–XII, VI–XVIII, and IX–XI.

The virtual failure of homoeologous chromosomes to pair appears to be due to suppression of pairing by chromosome V. When chromosome V is missing, hybrids of hexaploid wheat (genomic formula AABBDD) with amphidiploid *T. aegilopoides* × *Aegilops squarrosa* (AADD) show many microsporocytes with the equivalent of

the expected 14 bivalents (average over 12), but only rarely have as many as 13 bivalents (average about 8) when V is present. Hybrids of AABBDD with AA, which normally have only 2 to 7 pairs, have the equivalent of 5 to 13 pairs (average about 10) when chromosome V is missing. It seems likely that the acquisition of a mutation on chromosome V which decreases pairing intensity is the means by which hexaploid wheat has achieved diploid pairing. As demanded by this hypothesis, pairing is definitely less intense in hexaploid than in diploid wheat.

SERMAN, D. *See* HARTMAN, P. E., *et al.*

SERMONTI, GIUSEPPE, and GIORGIO MORPURGO. Chemio-Reactivation in *Penicillium chrysogenum*. *Istituto Superiore di Sanità, Rome, Italy.*

Diploid conidia of *Penicillium chrysogenum*, inactivated by a nitrogen mustard (HN-2), have been reactivated by $MnCl_2$ added to the plating medium. When the survival of the HN-2-treated conidia on the unsupplemented medium is superior to 0.02, the killing effect is almost completely suppressed on the $MnCl_2$-supplemented medium. Once the reactivated clones have started to grow on the $MnCl_2$-medium, they do not require $MnCl_2$ for further development. The reactivating effect of $MnCl_2$ is much less remarkable on haploid conidia than on diploid ones. No reactivation by $MnCl_2$ has been observed on conidia inactivated by ultraviolet or X-rays. After nine hours' incubation of the HN-2-treated conidia in a nutrient medium the efficiency of the reactivation has not been affected. $MnCl_2$ is also effective in reducing the rate of HN-2-induced somatic segregation from heterozygous diploid conidia, and in inhibiting the spontaneous segregation of HN-2-induced unstable clones.

SERMONTI, GIUSEPPE. *See* MORPURGO, GIORGIO.

SERRA, J. A. Two-dimensional Composite Nature of the Gene. *Institute of Zoology, Faculty of Science, Lisbon, Portugal.*

A series of facts relating to isoalleles and position effects and also recent results on pseudoalleles may be explained by the composite model of the gene which we developed several years ago (Serra, Bol. Soc. Broter. 2s *19*: 327–369; Portug. Acta Biol. (A), 1949). The gene has been represented as a composite, along the chromonema, of one to several segments of chromonema (nemameres). Gene mutations and position effects of the mimic type consist in changes in the contacts between successive segments, respectively within and between the genes. Other position effects are of the pure type, not mimic to known gene mutations. Pure position effects are potentially new genes, and repeats should provide the most frequent process of their origin.

This model also considered the composite nature of the gene according to its diameter. However, it applies only to macro-organisms and may need to be simplified when applied to micro-organisms. To draw a conclusion from the diameter of

one chromoneme, it is certain that each gene is composed of several fibrils. Electron micrographies clearly show about 6–8 orders of fibrils, down to about 40 Å (diameter of two double polynucleotide or polypeptide chains), each fibril formed of two of the next inferior order. So-called gene conversion or transmutation may be explained by the sorting-out or cross-sorting of different fibrils within a gene, when cross-over takes place. If, to simplify, we suppose there are only two double fibrils in each chromoneme, then a heterozygote would normally be *aa.aa/AA.AA*. If by partial mutation $a \rightarrow A$, or by a former somatic cross-over or asynchronous reduplication, it becomes *AA.AA/Aa.aA*, a 3:1 tetrad of $AA+AA+AA+aa$, or a 4:0 of $AA+AA+Aa+Aa$ may result, as found in several cases. In meiosis this process should be linked with, but independent of, crossing-over. Sorting-out may also explain negative chromatid interference, somatic cross-over and some cases of gene instability.

SHAPIRO, N. *See* BREWBAKER, J. L.

SHARMA, ARUN KUMAR. Aesculin: Its Use in Detecting Double Fertilization and Chromosome Structure of Various Organs of Plants. *Cytogenetics Laboratory, Botany Department, Calcutta University, Calcutta, India.*

To cytologists and geneticists, it often becomes imperative (especially after cross breeding) to determine the occurrence of fertilization and double fertilization leading to endosperm development in higher plants. Pollen tube smear technique is not suitable and other classical cytological methods are often too time-consuming for a breeder.

Aesculin has already been used in the clarification of chromosome structure of roots and leaves (Sharma and Sarker, 1956; Sharma and Sharma, 1957). It is now possible to employ this chemical effectively in the clarification of chromosome structure of endosperm and embryo tissue. With the aid of the following schedule, the nature of the endosperm—its triploid constitution following double fertilization—can be demonstrated in four hours or less. Ovules are treated in approximately 3% aesculin solution for 3 hours at a temperature of 10–12°C. The ovules are then fixed in acetic-alcohol (1:1) for 30 minutes. The materials are next heated in a mixture of 2% aceto-orcein and N/HCL (9:1) for a few seconds. After ten minutes, they are smeared in 1% aceto-orcein solution. In such preparations the number of chromosomes is readily ascertained both in the endosperm and in the embryo, and chromosome morphology is clearly delineated. In the study of pollen grain chromosomes, the use of aesculin has also been found to be advantageous in that the least contraction of chromosomes in a perfectly scattered plate can be secured.

It has been shown that in the same individual the concentration of aesculin and the period of treatment necessary for the clarification of chromosome structure in different organs vary to a considerable extent. They are decidedly different for leaves, stem-tips, roots, ovules and pollen grains. This is an indication of the differential constitution of the cytoplasmic set-up of these various organs. It has been shown that aesculin causes viscosity changes in the cytoplasm.

SHEPPARD, P. M. *See* CLARKE, C. A.

SHULTZ, FRED T. *See* ANDERSEN, A. C.

SIEGEL, ALBERT, A. NORMAN, and W. GINOZA. The Inactivation of Tobacco Mosaic Virus by Ultraviolet Light. *University of California, Los Angeles, U.S.A.*

Strain U1 of tobacco mosaic virus is 5½ times more resistant to inactivation by ultraviolet light than strain U2. The infectious nucleic acids derived from these strains, however, are both equally sensitive to ultraviolet light. Moreover, the nucleic acids have a sensitivity equal to that of the intact U2 strain. Thus the genetic material of the intact U1 strain is protected from the damaging effects of ultraviolet light. The presence of water is necessary for this protection. When the U1 strain is irradiated dry, it has the same sensitivity as the U2 strain. Drying of the U2 strain does not affect its sensitivity. The above data were obtained with irradiation of wavelength 254 mμ. When the two strains are irradiated at 226 mμ, they have approximately the same sensitivity. At this wavelength, strain U1 is approximately five times more sensitive than at 254 mμ, while strain U2 is almost equally sensitive at the two wavelengths. At wavelength 254 mμ, the nucleic acid component of the virus accounts for approximately 80% of the total absorption of the virus, while at 226 mμ the nucleic acid absorbs only a minor fraction of the total energy absorbed. It is postulated that absorption of energy either by the nucleic acid or by the protein portion of the virus can lead to inactivation of the U2 strain. Absorption of energy by the nucleic acid of the U1 strain is relatively ineffective and the inactivation of this strain is due mainly to absorption of energy by the protein. When inactivation is a result of absorption of energy by protein, the energy is apparently transferred to the nucleic acid and the nucleic acid becomes damaged. This is demonstrated by the fact that nucleic acid extracted from irradiated U1 strain is found to be inactivated to the same extent as the parent virus.

SILVESTRONI, E. *See* SINISCALCO, MARCELLO, *et al.*

SINGLETON, W. RALPH, and ALAN L. CASPAR. The Blandy Radiation Field for Inducing Mutations in Plants. *Blandy Experimental Farm, University of Virginia, Charlottesville, Va., U.S.A.*

The Blandy radiation field, or pit, is designed to give short rather than long exposures of chronic radiation. Plants are grown in pails and moved to the radiation area which consists of a paved circle 30 feet in diameter, shielded by a concrete retaining wall and an earthen embankment. The 125 curie Co^{60} machine is located in a dry well in the center of the field. The source is raised by remote control through a stainless steel pipe to the operating position. A "sky shine shield" consisting of three tons of concrete blocks on an oak table limits the radiation upward that would be reflected to earth by particles in the atmosphere.

The Co^{60} machine was loaned to us by the Division of Biology and Medicine of the Atomic Energy Commission. Cost of the field exclusive of the machine was approximately $5,700. (Research work was supported by grant from the Atomic Energy Commission, A. E. C. Contract No. AT-(40-1)-2071.)

Siniscalco, Marcello, T. Bianco, G. Montalenti, and E. Silvestroni. Effect of Thalassemia on the Pattern of Some Physical Measurements. *Istituto di Genetica, Università di Napoli, Naples, Italy.*

By studying the distribution of some physical measurements (stature, weight, cephalic indexes, etc.) in a sample of families derived from a thalassemic area (Berra, District of Ferrara, Italy) some evidence is put forward in favour of the conclusion that the thalassemic gene produces some direct effect on the pattern of these measurements. Apart from considerations relating to the genetic problem of thalassemia, the authors stress some general implications of these observations. The possibility that a single gene difference might alter more or less directly the pattern of some classical physical measurements seems to be an important point for the physical anthropologist to bear in mind.

Sinotô, Yosito. Graft Experiments on Eggplants. *Department of Biology, International Christian University, Mitaka, Tokyo, Japan.*

Graft experiments have been carried out on several varieties of eggplant since 1951, in order to re-examine the results of the Lysenkoists who obtained vegetative hybrids. The results of the present work were published in part in 1955. (The Japanese names of the varieties are given.)

(1) Kantôao (KA, blue fruits) was grafted on Sinkuro (B, black fruits). Twenty were successfully grafted of which 16 bore fruits in the branches of the scions. In 9 plants the fruits were blue, while in the other 7 plants they were black. (2) Sinkuro (B) was grafted on Kantôao (KA). Of 4 grafted plants 2 showed that the fruits on the stock (KA) were all blue, while in the other 2 plants bodies, leaves and fruits of the stocks (KA) all changed to black. (3) The first generation raised from seeds obtained by selfing in the blackened fruit borne on the scion (KA) showed segregation into the two types of plants: 25 blues and 10 blacks.

The author has tried to interpret these data hypothetically from the standpoint of biochemical and physiological genetics, especially by means of the concept of actant which has been advocated by the author since 1938 (Kagakugahô *27*, 1938, 1945; Novant' anni del leggi mendeliane, Rome, 1955; Kagaku *25*, 1955).

Sisken, Jesse E. The Synthesis of Nucleic Acids and Proteins in the Nuclei of *Tradescantia* Root Tips. *City of Hope Medical Center, Medical Research Institute, Duarte, Calif., U.S.A.*

An attempt has been made to correlate the synthesis of nucleic acids and some proteins of the nucleus with events of the mitotic cycle. By means of radioisotopes and the autoradiographs, the incorporation of specific precursors into nuclei of *Tradescantia* root tips was determined. Interphase, the period between division stages, may be divided into three sub-phases. In the first, the nucleus enlarges but does not incorporate tritiated thymidine. Since thymidine is a specific label for deoxyribonucleic acid (DNA), growth of the nucleus must occur without the synthesis of DNA. In the second sub-phase, which occurs when the nucleus has reached a certain size, incorporation of thymidine occurs without further increase in size. Data derived from cells in stages of division show that there is a third phase

between the period of DNA synthesis and prophase which requires about eight hours. To obtain information concerning the time of synthesis of ribonucleic acid (RNA), the incorporation of Cl4-orotic acid, a precursor of both types of nucleic acid, was studied. The uptake of isotope into both RNA and DNA was determined by studying two comparable sections of the same nucleus after RNA had been removed from one by hydrolysis in N HCl at 60°C for 7 minutes. Data indicate that at the time of synthesis of DNA, there is little or no synthesis of RNA and that most of the RNA of the nucleus is synthesized just prior to the synthesis of DNA. There is a secondary period of RNA synthesis following DNA synthesis. The synthesis of nuclear proteins which are not removed by the Feulgen hydrolysis was studied using Cl4-lysine. The data indicate that protein synthesis probably occurs in all stages of the cell cycle.

SKALINSKA, MARIA. Some Attempts to Obtain Hybrids between *Aquilegia* and *Isopyrum*. *Institute of Plant Anatomy & Cytology, University of Krakow, Krakow, Poland.*

The degree of relationship between some genera may sometimes be estimated from their crossability. *Aquilegia* and *Isopyrum* are regarded by taxonomists as closely related genera. They have the same number of chromosomes ($2n = 14$) and they belong to the group of Ranunculaceae with very small chromosomes (Gregory, 1941). In order to test their crossability, the most primitive species of *Aquilegia, A. ecalcarata* Maxim. was chosen. The first stages of development after cross-pollination seemed promising. Germination of pollen tubes of *Isopyrum thalictroides* L. on the styles of *Aquilegia*, although inferior to that after selfiing, was observed. Fertilization occurred 3 to 6 days after pollination and was followed by the development of the endosperm. After 9 days some apparently normal ovules contained 350 to 437 free endosperm nuclei and in some others the transition to the cellular state was observed. The first division of the zygote occurred 5 to 7 days after pollination. The embryos however attained only the stages of 3 to 8 cells (exceptionally, 14 cells) before the beginning of abortion. In most ovules also the endosperm has in later stages an impaired growth. Its breakdown is not bound to a definite stage; it takes place in various stages of development. The poor viability of the hybrid embryo is not regularly correlated with that of the endosperm; it seems to result rather from its genetic constitution. During experiments in three successive years only non-germinating seeds with well-developed seed-coat and sometimes well filled with endosperm but without embryo were obtained.

SLATIS, HERMAN M. An Analysis of Inbreeding in the European Bison. *Argonne National Laboratory, Lemont, Ill., U.S.A.*

In connection with the study of inbreeding in man, a survey was made of the possibility of obtaining information about inbreeding in other large mammals. It was hoped that inbreeding could be studied through the use of zoo records of mammals from wide-ranging, numerous species, such as giraffes or zebras. Inquiry indicated that such records are available in only a few cases. However, the existence of excellent data on the European Bison was pointed out.

At the present time there are almost 200 living European Bison and individual

records exist for about 400 additional specimens, including almost all the animals kept in captivity within the preceding 60 years. All the living animals are at least the third generation in captivity and a few can be traced in some lines through 10 generations. The living bison trace back to a set of 17 ancestors, of which only one was born in captivity.

The coefficient of inbreeding, F, has been calculated for all of the animals on the assumption that the 17 original animals were unrelated to each other. Mortality is quite similar at all levels of inbreeding, with little or no indication of early death being correlated with inbreeding. F may not be a satisfactory index for this study. An alternative index has been developed, *ll, the likelihood of death if a given ancestor possessed a recessive lethal gene heterozygously*. This index also shows little correlation with early death.

Given sufficient data, the comparison of inbreeding as measured by F and by *ll* will provide information on the roles of homozygosity for many loci as opposed to homozygosity for specific lethal loci in the reduction of viability that is noted in inbred populations.

(This work was performed under the auspices of the U. S. Atomic Energy Commission.)

SLIZYNSKI, B. M. Chiasmata in Female Mice. *Institute of Animal Genetics, Edinburgh University, Edinburgh, Scotland.*

At the present stage of technique it is impossible to count chiasmata in oocytes at the diplotene stage. The data have been collected on chiasma frequency of 50 oocytes at metaphase stage. In comparison with analogous data for spermatocytes, females have more chiasmata per nucleus and per bivalent than males. The mean chiasma frequency per bivalent is in females 2.03 and in males 1.71. Estimated diplotene map length of the whole complement for females is about 2,130 cM, i.e., about 10% more than the 1,896 cM long diplotene map of the male hybrid animals. It is possible that cytological differences in behaviour of sex bivalents in females and in males may be correlated with the sexual dimorphism in the frequency of chiasmata and recombination.

SMITH, BEN W. Polyploidy, Hybridization, and Sexuality in Dioecious *Rumex hastatulus* and *Rumex paucifolius*. *Genetics Faculty, North Carolina State College, Raleigh, N.C., U.S.A.*

The cytogenetics of sex determination in circumboreal *Rumex acetosa* L. and its allies is well known: $^{X}/A$ balance; $2n = 14,15$; X versus Y_1Y_2 in the heterogametic males. The two American species of the subgenus Acetosa are also dioecious, but differ markedly from *R. acetosa* and from each other both in chromosome number and morphology. *Rumex hastatulus* Baldw. is an annual weed of the Southeastern Coastal Plain. Females are homogametic ($n = 3 + X$); males are heterogametic ($n = 3 + X$ or $3 + Y_1Y_2$). The combination $3A + 2X + Y_1Y_2$ produced a unisexual male; $4A + 3X + Y_1Y_2$ resulted in males and male intersexes. This suggests an X/A balance with a critical value near 0.75, but does not exclude the possibility that the Y-chromosomes influence sex determination. *Rumex paucifolius* Nutt., a perennial subalpine of the western mountains, includes at least two chromo-

some races, $2n = 14, 28$. Chromosome morphology and meiotic behavior reveal the autopolyploid nature of the 28-chromosome forms. The diploid is known from a single collection. We have not observed heteromorphic sex chromosomes.

Reciprocal crosses between *R. hastatulus* and *R. paucifolius* ($2n = 28$) have both produced F_1 hybrids. The hybrid population was dioecious; no intersexes occurred. Of the 51 hybrids 6 were vigorous, "neuter" plants which failed to produce any flowers or flower parts. With *R. paucifolius* as pollen parent all hybrids received 18 chromosomes. Sex was determined by heterogamety in *R. paucifolius*. Following the reciprocal cross, female hybrids contained 18 chromosomes; 14 from *R. paucifolius*, 4 from *R. hastatulus*. The 19-chromosome males received 5 chromosomes ($3 + Y_1Y_2$) from *R. hastatulus*. At metaphase I in PMC's, there are 7 bivalents and 4 or 5 univalents. As the *R. paucifolius* bivalents disjoin, the *R. hastatulus* univalents divide. The daughter chromosomes usually reach the poles. From thousands of florets, we obtained only 50 viable F_2, backcross, and open-pollinated seed. In the resulting plants, the parental chromosomes occurred in various new combinations which have been correlated with sex expression.

SMITH, HAROLD H. Limits and Consequences of Multiple Allopolyploidy in *Nicotiana*. *Biology Department, Brookhaven National Laboratory, Upton, Long Island, N.Y., U.S.A.*

A multiple allopolyploid *Nicotiana bigelovii-debneyi-tabacum* ($n = 72$) was produced by crossing the amphidiploid *N. debneyi-tabacum* ($n = 48$) with *N. bigelovii* ($n = 24$) and doubling the chromosome number with colchicine. Cultures were continued by self-pollination and selection for three distinct races through the S_{10} generation. Each race has become established at a different high aneuploid number of chromosomes ($n = 50$ to 60) and irregularities in meiosis persist. Pollen abortion has been reduced to 3.7% in race A, 15.7% in race B, and 8.5% in race C. Of 15 plant characteristics measured, race A differs from B in 10 and from C in 8; races B and C differ in 9. The races were also characterized in terms of crossability and morphological comparisons with the parental species. It has been shown, then, that higher allopolyploidy may give rise to populations with novel aneuploid numbers, high fertility, and distinctive morphologies. (Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.)

SMITH, JIMMIE B., and TETSUO MATSUI. Unusual Origin of a *Drosophila* Gynandromorph. *Botany Department, University of Hawaii, Honolulu, Hawaii.*

A bilateral gynandromorph was discovered in *Drosophila* among the progeny of a cross between a balanced lethal female of genotype $\frac{Bl\ +}{+\ Cy}$ and a male homozygous for the recessive, hairy. The fly was a typical sexual mosaic such as are presumed to arise by X chromosome elimination in an early cleavage division. However, this fly was also bilaterally mosaic for the two dominants of the balanced lethal parent, which are located on the second autosome. The Bl (short bristle) trait was associated with the left or male side of the fly, the Cy (curly wing) trait with the right

or female side of the fly. Mosaicism for both sex-linked and autosomal traits almost certainly means that this fly resulted from a simultaneous fertilization of a binucleate egg with unlike sperm. That silkworm mosaics arise in this way has been ingeniously demonstrated, but only four such cases have been reported for *Drosophila*. Of the four, this fly seems most clearly to prove that gynandromorphs in the fruit fly can arise by other means than by X-chromosome elimination, contrary to the impression given by most texts on genetics.

SMITH, LOIS J. *See* RUSSELL, ELIZABETH S.

SMITH, S. G. *See* MANNA, G. K.

SMITHIES, OLIVER. The Serum β-globulin System in Humans. *Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada.*

Two well-recognized examples of polymorphism in human proteins (the serum haptoglobins and some serum γ-globulins) appear neither to be associated with red cell blood groups nor to lead to metabolic abnormalities. The involvement of genetic factors in the control of both these systems has been established (Smithies and Walker, 1955 and 1956; Grubb, 1956). The present paper summarizes the evidence for a further comparable example of protein polymorphism in human serum β-globulins.

The presence of a previously unrecognized β-globulin D was demonstrated by two-dimensional starch-gel electrophoresis (Smithies, 1957) in the sera of 2 New York negroes (49 tested) and 5 Australian aborigines (23 tested). In the sera of several hundred Canadians previously examined β-globulin D was not observed and β-globulin C was quantitatively the predominant β-globulin. Sera from more Australian aborgines and their families have been studied and a third β-globulin type has been observed in which β-globulin D is present, but β-globulin C is essentially absent. The results of these studies suggest that the presence or absence of β-globulins C and D in the serum is controlled by 2 autosomal alleles (β^C and β^D) with no dominance (Horsfall and Smithies, 1958).

Evidence has also been obtained for a third allele (β^B) at the β-globulin locus in whites (Smithies, 1958). Protein patterns in which β-globulins B and C are present in apparently equal amounts have been seen with sera from 5 donors (425 tested) at the blood bank of the Toronto General Hospital. (In the usual patterns β-globulin C is present in much greater amounts than β-globulin B.) Family studies suggest that these 5 persons are heterozygous for the genes β^B and β^C.

No correlation between the β-globulin types and the ABO, Rh, MNS, P, Kell, Duffy and Lewis blood groups has been observed.

SNELL, GEORGE D. Histocompatibility Genes in the Mouse. *Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A.*

This exhibit shows how individual histocompatibility genes in the mouse have been identified. Several techniques are illustrated. The use of tumor cell suspensions pre-

pared in the cytosieve permits the administration of a controlled dose. Mice are easily immunized with a cell suspension of thymus from the tumor donor. These two techniques make possible the demonstration of "weak" histocompatibility differences with transplantable tumors, which otherwise sometimes respond only to "strong" differences between donor and host. The most valuable genetic techniques are based on the use of coisogenic strain pairs (CSPS), i.e., strain pairs essentially identical except for a difference at a single histocompatibility locus. A chart shows how these are produced.

Three histocompatibility (H) loci are now known. *H-1*, in the first linkage group, shows about 7.1% crossing over with albinism. It may be that the *H-1* antigen is absent in skin, since skin grafts (Dr. Stevens) take successfully between members of a CSP differing at *H1*. Though "weaker" than *H-2*, *H-1* imposes a fairly effective barrier to the transplantation of leukemias. *H-2* was first identified by Dr. Gorer as a blood group locus. It plays a dominant role in all histocompatibility phenomena in the mouse. At least 16 alleles are now known, each of which determines a group of (8 or more) antigenic factors or components. Crossing over between the components has been demonstrated independently by Drs. Gorer and Allen. *H-2* is thus actually a system of pseudoalleles. It is in linkage group IX about 5 units from *Fu* (Allen). *H-3* is in linkage group V, about 9.8 units from *a* (this estimate may be high). Though probably a less complex system than *H-2*, there is already evidence for 4 alleles. Skin grafts between members of a CSP differing at *H-3* survive about 3-4 weeks as compared to 8-10 days for strain pairs differing at *H-2*.

SOBELS, F. H. Rates of Forward and Reverse Mutation Induced by Mustard Gas in *Drosophila*. *Institute of Genetics, State University, Utrecht, Netherlands.*

A study on the frequency of reversion of the mutant forked^{3n} of *Drosophila* after treatment with mustard gas was combined with tests for recessive visible mutations by using Muller's "Maxy" stock. Treated cv f^{3n} males were mated during four days to y ac pn w rb cm ct^{6} sn^{3} oc ras^{2} v dy g^{2} f od car sw /y sc^{S1} B f In 49 v females. Daughters suspected of carrying a mutation for one of the marker genes in the paternal X-chromosome were subjected to further genetic tests. The over-all mutagenic effectiveness of the mustard dose was measured for each experiment separately by sex-linked lethal tests. The weighted mean frequency of sex-linked lethals for 6 experiments under consideration is 11.2% ($n = 3{,}777$). After 5,000 r X-irradiation the same stock yielded 13.7% ($n = 2{,}252$) lethals. The genetic effects of the mustard dose used therefore correspond roughly to that of 4,000–4,500 r X-rays. In a total of 16,432 gametes the following numbers of male viable and fertile mutations were obtained: 7 ruby, 6 white, 5 garnet, 3 carmine, 2 prune, 2 singed. In 33,098 gametes 3 yellow, 3 vermilion and 7 reversions (5 complete, 2 partial) of f^{3n} to wild type were scored. Four eye color mutations (3 rb and 1 g) were isolated from females with mosaic gonads, indicating delayed effect of the mutagenic treatment. Out of 7 forked reversions 3 were likewise characterized by delayed appearance. All reversions segregate in crosses as sex-linked dominants. Tests to determine whether closely-linked dominant suppressors are involved are not yet completed. The spontaneous rate of back mutation of f^{3n} is according to Green (1958) 1/20,000. After 4,000 r X-rays Green (unpublished) obtained 38 f+ in 115,774 chromosomes and after gamma irradiation from Co^{60} 10 f+ in 65,206 chromosomes. It would seem so far that the rate of reversion induced by mustard gas falls in the same range as that produced by ionizing irradiation. The material for recessive visibles is still too small

for any definite conclusion. With the possible exception of the ruby and white loci where Valencia and Muller (1949) reported 1/8,000 after 4,000 r X-rays, a comparison with their X-ray data does not suggest an entirely different response to the action of mustard gas. Because of delayed effects, comparisons of the mutagenic effectiveness of mustard gas with that of X-irradiation inevitably involve an underestimate for the chemical.

I want to express my appreciation to Dr. M. M. Green for the use of his unpublished data, and to Dr. I. I. Oster for supplying me with some of the stocks. This work was supported by the Health Research Council T.N.O.

(G. J. O. Jansen, J. Ringelberg and S. C. M. Schouten collaborated on parts of this project.)

Sokal, Robert R. Selection for Differences in Pupation Site of *Drosophila melanogaster*. *Department of Entomology, University of Kansas, Lawrence, Kan., U.S.A.*

Drosophila melanogaster reared under controlled conditions in 6 dram shell vials containing cornmeal-molasses-agar medium will pupate at various places from the center of the medium surface to positions on the wall of the vial above the medium. Pupation site was observed in vials into which 10 eggs had been placed and from which optimally 10 pupae resulted. Since there was varying mortality of eggs and larvae and it was found that increased density lowered the percentage of peripheral pupation, pupation site readings had to be corrected for a constant density.

Mass selection (on a combined individual-family merit basis) for differences in pupation site resulted in a peripherally pupating (PP) and a centrally pupating (CP) line. Response of the two lines was asymmetrical. The PP line responded immediately and strikingly with a plateau reached and maintained by generation 15, while the CP line reacted only gradually and not by very much. At generation 18 a maximum difference was attained when the PP line had 100% peripheral pupae while the CP line had only 6% such pupae. The differences declined somewhat during periods of relaxed selection but were quickly re-established when selection was renewed.

Fertility and fecundity declined during selection, but recovered when it was relaxed. The 2 lines were examined for differences in: length of larval period, sex ratio, resistance of larvae to DDT in the medium, and 14 morphological characters. Appreciable differences were found in all but the first two.

Considerable statistical analysis has been performed in the interpretation of these data. The character as measured in percentages did not lend itself to satisfactory analysis and had to be transformed to a probit scale. An analysis of variance and covariance components for various generations of selection was undertaken in order to separate genetic and environmental effects. Fluctuations were observed in the expression of the character which appear to be due to unaccountable environmental variations. A number of analytical methods for dealing with such a situation are discussed.

Crosses and chromosome assays demonstrated that pupation site is a multifactorial character and have permitted estimates of the contributions of the various chromosomes to the expression of the phenotype. Evidence is presented that selection in the two opposite directions has affected different genic systems.

(Studies were aided by contract with Office of Naval Research Nonr. 171 (00).)

SOKOLOVSKAJA, I. I. Metabolic Changes in the Fertilization Action of Domestic Mammals. *All-Union Institute of Animal Breeding, Moscow, U.S.S.R.*

Fertilization, in the broad sense, is a complex of physiological actions, which includes gametogenesis, sexual reflexes, insemination, and the proper fertilization or the union of gametes.

There is a unity of the specific metabolic action of fertilization and the general physiological reactions in organisms (oxidizing-reducing actions, the changes of colloidal conditions, selective processes, the changes of O_2 uptake, and so on). The investigation of the role in fertilization of some glucoproteins and the ferments which act on them initiates the study of albumen metabolism in fertilization.

The presence of hyaluronic acid was established in the zona pellucida of ova, in viscous matter of granulosa, and in the seminiferous tubules of testicles (in the Certoly simplast). After spermatozoa enter the zona pellucida, changes in the uptake of hyaluronic acid were observed. Histochemical and ferment reactions (mucicarmin, hexamethylen–tetramin silver complex, the ferment action on the zygotes) as well as the behaviour of radioactive sulfur (S^{35}), being included in organic matters (tiamin, cystin and others), show the presence of mucins in the zygote's zona pellucida and protective layer (which is formed in the oviduct), in the secretion of cervix of the uterus of some mammals and Cooper glands of hogs. The protective layer of the zygote consists of mucin and the source of this layer is a secretion of the oviduct's cells. In the uterus secretion of cows and sows and in the secretion of the rabbit's prostatic gland the ferment mucinase was found. This ferment provides specific changes of the colloid's conditions of mucins, in particular, the mucins of the cervical secretion of cows and ewes.

The richest source of the mucinase in nature is the prostatic gland's secretion in rabbits.

Mucinase is thermolabile. Dried at room temperature it does not lose its potency for years.

The addition of mucinase to the synthetic medium for semen (0.25 mg. per one cow) facilitates the spermatozoon's advance to the site of fertilization and so increases the possibilities of impregnation by 15% on the average.

SOLIMA, ANGELA. *See* NICOLETTI, BENEDETTO.

SPARROW, ARNOLD H. *See* CUÁNY, ROBERT L., *et al.*

SPIESS, ELIOT B. Relation between Karyotype Components of a Population and Adaptive Values of Gene Arrangements in *Drosophila persimilis*. *Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pa., U.S.A.*

It has been demonstrated (Spiess, 1957) that the karyotype combinations of chromosome III gene arrangements (*D. persimilis*), Klamath and Mendocino, which are rare in the wild habitat sampled, are approximately equal in adaptive value when competing together under laboratory conditions at 16°C. When the commonest arrangement (Whitney) competes with either KL or MD, on the other hand, fre-

quencies of WT-KL or WT-MD come to stable equilibrium. WT *vs.* KL gives $\hat{q} = 25\%$ KL and WT *vs.* MD gives $\hat{q} = 37\%$ MD approximately. Six experimental populations containing all three arrangements come to stable equilibrium after seven to ten generations at approximately 78% WT, 8% KL, and 14% MD. MD is higher than KL in all but one population at equilibrium, by 7–10%. These data corroborate the evidence obtained from two-arrangement populations that interaction of the WT arrangement brings about a constant inequality between KL/KL and MD/MD; and the order of relative superiority for karyotypes in three-arrangement populations agrees with that expected from the WT two-arrangement experiments. (This work has been done under Contract No. AT-(30-1)-1775 with the United States Atomic Energy Commission.)

SPOFFORD, JANICE B. Parental Control of Position-Effect Variegation. *Department of Zoology, University of Chicago, Chicago, Ill., U.S.A.*

The extent of variegation of eye pigmentation in male *Drosophila melanogaster* was found to depend not only on the genotype of the affected fly but on the genotypes of his parents. Variegation resulted from an insertion (*Dp* (w^+)) of a small region of the X chromosome including the w^+ locus into the centromeric heterochromatin of 3L. The X chromosome carried *w*. Various Y chromosome fragments were present singly in individual flies. The experimental flies were derived from crosses between stocks whose X, second and normal third chromosomes were rendered reasonably co-isogenic by use of the Muller-5; *Cy*; *Ubx/Xa* technique. Extent of variegation was estimated visually for both eyes of each fly and was measured photofluorometrically on two spots separated chromotographically from each head. When the w^+-bearing third chromosome in the sons was derived from a mother homozygous for it, the pigmentation in some of these males was 90 times as great as when the *Dp* (w^+) came from a heterozygous mother. This effect is more pronounced in the presence of certain Y fragments than of others. Whether the sex of a heterozygous parent contributing the duplication or the sex of the parent contributing the Y has an important influence on the extent of variegation in sons was also studied. Only a complex causal mechanism responsible for position-effect variegation at the w^+ locus could account for pre-fertilization influence of maternal genotype on an essentially biochemical trait developed late in pupation.

SPOFFORD, JANICE B. *See* BAKER, WILLIAM K.

SPRAGUE, LUCIAN M. The Inheritance of a Natural Antibody in Cattle. *U.S. Bureau of Commercial Fisheries* and *Scripps Institute of Oceanography, La Jolla, Calif., U.S.A.*

Natural antibodies are portions of the normal globulin moiety of the sera of many species of animals and have been recognized by serologists for over seventy-five years. Some of these fractions are recognized by their reaction with the red blood

corpuscles of the same or different species. Students interested in the origin of natural antibodies disagree as to whether natural antibodies are fundamentally a normal physiological property of the individual or species and therefore subject to genetic control or whether natural antibodies are immune responses to a generally similar environment and are therefore subject essentially to external environmental origins.

The discovery of a relatively rare antibody in the normal serum of cattle by Stormont (1951) and the pattern of its occurrence suggested that it might be subject to genetic control. This antibody is designated normal cattle anti-sheep O and referred to simply as anti-O. Since its discovery, 128 cattle have been recorded as containing demonstrable anti-O fractions in their sera. The pattern of the occurrence of anti-O antibodies is compatible with the hypothesis that anti-O is the product of a single dominant gene.

Two other properties influence the phenotypic expression of animals carrying the gene for anti-O. These two properties are the two soluble blood group substances found in the serum of some cattle. One of these described by Sprague (1957) has been designated Oc or cattle O substance. The other is cattle J substance described by Stormont (1949).

Anti-O is not detectable in the serum of cattle in which either or both of the J and/or Oc substances can be found. J-positive and/or Oc-positive animals may have J-negative, Oc-negative, anti-O offspring. The anti-O gene may therefore be regarded as an hypostatic dominant with regular autosomal segregation.

SRB, ADRIAN M. Different Expressions of a Nuclear Gene in Combination with Different *Neurospora* Cytoplasms. *Department of Plant Breeding, Cornell University, Ithaca, N.Y., U.S.A.*

A spontaneous mutant of *Neurospora sitophila* has an aconidial phenotype. When the mutant culture is crossed reciprocally with wild-type *N. sitophila*, the segregation pattern in either progeny indicates the aconidial characteristic to be based on a simple allelic difference from the wild type. The results are quite different when the aconidial *N. sitophila* is reciprocally crossed with a stock of *Neurospora* whose nuclear genome is essentially that of wild-type *N. sitophila*, but whose cytoplasm is SG-mutant cytoplasm from *N. crassa*. This latter stock has been obtained as the result of a series of nine back-crosses following an original interspecific cross where SG-*Neurospora crassa* was used as protoperithecial parent and *N. sitophila* was used as conidial parent. The back-cross series was such that *N. sitophila* was used as a recurrent conidial parent, while the protoperithecial parent was always an isolate from the preceding generation, carrying SG-*N. crassa* cytoplasm. When the reciprocal cross is made between the aconidial *N. sitophila* and the stock carrying an *N. sitophila* nuclear genome but SG-cytoplasm from *N. crassa*, the two progenies are as follows: (*a*) the progeny whose protoperithecial parent was aconidial *N. sitophila* show a one to one segregation for the aconidial characteristic; (*b*) the progeny whose protoperithecial parent carried SG-cytoplasm from *N. crassa* typically are all conidiating cultures. That the nuclear gene for aconidial is represented among this latter progeny is shown by crosses where progeny cultures are crossed back onto *N. sitophila* cytoplasm. About half of such crosses show segregation for aconidial. The results seem best explained on the basis of differential interaction between a particular nuclear gene and different cytoplasms.

STADLER, JANICE, and JOHN W. GOWEN. Implication of Genetics to Radiation Effects on Quantitative Characters. *Genetics Department, Iowa State College, Ames, Iowa, U.S.A.*

The quantitative characters, weight and growth, natural and acquired disease resistance, fertility, litter size and frequency, life span etc. are utilized in our laboratory to detect and evaluate effects of different types of radiation. The genetic variability of the mouse population is broken up into discrete units having reproducible characteristics through the use of some ten inbred lines, their crosses, F_2's and backcrosses. Under conditions of only natural irradiation, these lines have shown differences in all of the quantitative characteristics listed above. Each of these attributes, except acquired immunity, is affected by whole body irradiation of X-rays and gamma rays of cobalt 60. The irradiation effects are detrimental to the exposed individuals. They show genotype and irradiation dose dependence. The differences extend to body regions as well as to the body as a whole. In terms of the interactions between genotypes, irradiation treatments and regional body differentiations, the results indicate that the effects largely depend on the individual cells. The large decreases in resistance to mouse typhoid, *Salmonella typhimurium*, when all cells were exposed to irradiation, over those expected from the summed contributions of the individual parts supports the conclusion that all body cells retain most of their initial genetically controlled ability to resist disease invasion even when they differentiate into specialized tissues. (This work has received assistance from Contract No. AT(11–1)107 from the Atomic Energy Commission.)

STADLER, JANICE. *See* FUNG, SUI-TONG.

STAIGER, HANS R. The Chromosomal Structure of Hybrid Populations of *Thais lapillus*. *Mergenthaler Laboratory for Biology, The Johns Hopkins University, Baltimore, Md., U.S.A.*

Two ecologically differentiated forms with the haploid numbers 13 and 18 chromosomes occur in the intertidal snail species *Thais* (= *Purpura*) *lapillus*; their numerical chromosomal dimorphism is due to five structural changes of the "centric fusion" type. The two forms interbreed freely where they come into contact in adequate habitats, forming hybrid populations with all possible degrees of chromosomal heterogeneity. An analysis of individual relative frequencies of chromosomes in populations over the whole range of heterogeneity demonstrates a numerical differentiation between the 5 chromosomes involved, revealing a definite chromosomal organization of the hybrid populations. The frequency data on the 243 ($= 3^5$) chromosomal constitutions, which may be formed on the basis of the numerical dimorphism, contradict heterosis and balanced polymorphism as a mechanism for stabilization of the chromosomal polymorphism. The data suggest a stabilization mechanism on the basis of inter-chromosomal interaction which favors individuals with complete and intact sets of dimorphic chromosomes of either form, while individuals with both sets combined from chromosomes of different origin are adaptively inferior.

STALKER, HARRISON D. Sex Frequencies in *Drosophila paramelanica*. *Department of Zoology, Washington University, St. Louis, Missouri, U.S.A.*

An X-chromosomal, male-limited sex-ratio trait similar to those known in other Drosophila species has been found in *Drosophila paramelanica*. Males hemizygous for the "sex-ratio gene" produce almost entirely X-bearing sperm, and thus daughters and very few sons. Approximately half of the sons that are produced by such males are from nullo-XY sperm and are thus XO and sterile. All "sex-ratio" X-chromosomes analysed carry at least two short inversions in one arm and two in the other, and show little crossing-over with the normal X-chromosome in heterozygous females. Studies made so far indicate that there are at least two kinds of sex-ratio genes and at least two genetically different kinds of Y-chromosomes in nature. The "northern" sex-ratio gene SR^N is effective with the "northern" Y-chromosome Y^N, but is suppressed when in combination with the "southern" Y-chromosome Y^S. The "southern" sex-ratio gene SR^S is expressed when present with either Y^S or Y^N. No morphological differences have been discovered between the two kinds of Y-chromosomes. Although in general SR^S and Y^S are found in southern populations and SR^N and Y^N to the north, some southern populations, as in Missouri and Indiana, have mixtures of Y^S and Y^N. In addition to the possible suppression brought about by the *Y-SR* interactions, most populations, northern and southern, have autosomal factors which may modify or completely suppress the *SR* genes.

STEHR, G. On the Determination of Polymorphism by an X-Chromosomal-Autosomal Balance. *Forest Insect Laboratory, Sault Ste. Marie, Ontario, Canada.*

Results of studies on the spruce budworm, *Choristoneura fumiferana* (Lepidoptera), lead to the general hypothesis that sex-controlled polymorphism in either or both sexes is determined by a balance between X-chromosomal and autosomal heteroallelic loci, similar to the balance mechanism that determines sex itself. On this basis polymorphism is interpreted as genetically akin to intersexuality.

STEIN, WERNER. *See* LASKOWSKI, WOLFGANG.

STEINBERG, ARTHUR G. Blood Group Studies on an Isolated Human Population. *Western Reserve University, Cleveland, Ohio, U.S.A.*

This is a preliminary report of a religious sect, the Hutterites, living in the Dakotas and Montana in the United States, and in Manitoba, Alberta and Saskatchewan in Canada. The study is designed to be an intensive investigation of population genetics based on data collected during a detailed medical-genetic investigation of the Hutterites.

The data were drawn from 3 of approximately 100 communal colonies forming the sect. Each colony consists of 100 to 175 individuals living communally on a large farm (3,500 to 5,000 acres). In all 443 Hutterites in about 50 families, with 15 surnames among them, settled into 4 communal colonies in South Dakota. Of

these 2 combined leaving 3 colonies, from which the present colonies are derived. Before 1900, another half-dozen Hutterite families joined the colonies. One new surname was added and one lost. Present-day Hutterites are all descendants of these people. The maximum sample of alleles at each locus in the population which gave rise to the present Hutterites is about 200 (50 families $\times$ 4); the minimum is about 32.

It is possible to trace the pedigree of every Hutterite who ever lived in a colony back to ancestors who settled in one of the original colonies in the United States.

When the population in a colony becomes too large, new land is purchased, and a group of families is chosen by lot to develop the new farm. Clearly this method of selecting individuals for the new farm provides an excellent sample for studying genetic drift. The Hutterites are divided into three sub-sects, one derived from each of the original three surviving colonies. There is essentially no marriage between individuals from different sects, thus providing further opportunity for studying genetic drift.

The blood group data indicate large variation in blood group and blood type frequencies among the three colonies studied, which can be explained most simply on the basis of drift.

STEINER, ERICH. Some Observations on Incompatibility Alleles of the Complex-Heterozygotes of *Oenothera*. *Department of Botany, University of Michigan, Ann Arbor, Mich., U.S.A.*

Earlier studies have demonstrated the presence of incompatibility alleles in the alpha complexes (those transmitted through the egg) of the *biennis* group 1 of *Oenothera*. Evidence of incompatibility alleles in the alpha complexes of races belonging to other phylogenetic groups can be obtained by producing hybrids combining these alpha complexes with the alpha *biennis* group 1 complexes. Self-incompatibility of such hybrids indicates the presence of incompatibility alleles. A survey currently in progress shows that the alpha complexes of at least four phylogenetic groups of complex-heterozygotes in addition to the *biennis* group 1 possess incompatibility alleles. In the race *Heber* results indicate that the *S* allele may have undergone mutation to an S_f or self-fertile form, at least in one plant. The data which are available at present support in general the assumption that each race carries a different *S* allele, although critical tests of the identity of alleles in the different races have not been completed. Preliminary tests with self-incompatible alpha·alpha hybrids whose chromosome numbers have been doubled indicate that the incompatibility reaction in the tetraploid is the same as in the diploid.

STEINITZ-SEARS, LOTTI M. The Structure of Chromosomes in the Female Gametophyte of *Triticum vulgare*. *University of Missouri, Columbia, Mo., U.S.A.*

A stain squash method for the study of the embryo sac mother cell and female gametophyte (Darlington and LaCour, The Handling of Chromosomes, 1950) has been adapted to wheat. Under the dissecting microscope ($\times$ 90) after Feulgen staining *in toto*, a clear distinction can be seen between outer integuments (diploid), inner integuments (diploid) and female gametophyte (haploid). In agreement with studies by Golinski in 1893 it has been found that the antipodal cells divide at least

three times in this species. Following the period of mitosis, they undergo a period of endomitotic divisions and growth until bundles of chromosomes, not unlike the salivary chromosomes of the Diptera, are found, but there is no fusion of centromeric regions into a common chromocenter or magma. As do salivaries, the antipodal cells eventually degenerate (similar data from other plants are given by Duncan and Ross, J. Hered. *41*: 259–268; Tschermak-Woess, Chromosoma *8*: 637–649).

The giant antipodal chromosomes of wheat are suitable for detailed studies of chromosome structures, such as heterochromatin, nucleolar organizing regions, centromeres and terminal chromosome sections. Megasporogenesis is being correlated with the developmental stages of the microspores in anthers of the same floret. As in other plants where megasporogenesis has been studied, it takes place later than microsporogenesis, approximately one week later in this species. Pachytene preparations are also being studied.

STERN, CURT. The Ratio of Monozygotic to Dizygotic Affected Twins and the Frequencies of Affected Twins in Unselected Data. *Department of Zoology, University of California, Berkeley, Calif., U.S.A.*

The significance of comparisons between the frequencies of monozygotic and dizygotic twins concordant for specific traits depends on the methods of ascertainment. With complete ascertainment in populations with twin material unselected except for the presence of a given trait, it has been assumed (1) that the ratio of monozygotic to dizygotic pairs is that of the general population, and (2) that the relative frequency of twins to singly born individuals is also like that in the general population. It will be shown that these assumptions are not valid.

Under the conditions stated (1) the ratio of ascertainable monozygotic to dizygotic twin pairs lies between one-half and one of the ratio in the general population, and (2) the ratio of all ascertainable twin pairs to the total number of individuals lies between one and two times of that found in the general population. Formulae will be derived which relate the frequencies of ascertainable twin pairs to the frequencies, from "suitable" parents, of the "affectable" genotype and to its penetrance. The calculated expectations will be compared with some observed values.

STERN, HERBERT, and T. S. FOSTER Some Intracellular Factors Governing DNA Duplication in Anthers of *Lilium longiflorium*. *Science Service, Canada Department of Agriculture, Ottawa, Canada.*

Chromosome duplication is associated in its primary stages with a pronounced shift in certain elementary metabolic processes of the cell. It will be shown that among such processes are those leading to the formation of low molecular weight deoxynucleosidic compounds. There is an obvious relation between the accumulation of soluble deoxynucleosidic substances and the subsequent synthesis of DNA in the sporogenous cells of the anther. A number of factors in the anther appear to be involved in this relationship. The possibility will be considered that the breakdown of tapetal cells furnishes DNA fragments which are used in later syntheses. The accumulation in the anther of the reducing compounds, glutathione and ascorbic acid, during periods of DNA synthesis, will also be discussed in terms of the genesis of deoxyribosides.

STEVENS, LEROY C. *See* BERNSTEIN, SELDON E.

STICH, H. F., and J. K. NAYLOR. Variation of the DNA, RNA and Protein Content of Specific Chromosome Regions during Development. *Department of Cancer Research, University of Saskatchewan, Saskatoon, Sask., Canada.*

Quantitative cytochemical studies of the basic mechanism of the "puffing" of particular chromosome regions, associated with chromosomal activity, were undertaken on the chromosomes of the salivary glands of the *Glyptotendipes* (Chironomidea) whose extraordinary size in an experimentally stretched condition enables the determination of DNA in single bands and in chromosome regions in which a puff is present or absent. The substances produced on the puffed regions were analyzed using cytochemical reactions for proteins, arginine and RNA.

Quantitative measurements made by the two-wave length method of Patau permitted the determination of the DNA (Feulgen) content of heterogeneous structures with less than 3% error. Similar measurements of chromosome regions treated with the Millon-reaction, the α-naphthol reagent (Leish and co-workers, 1957) and stained with fast green (Alfert and Geschwind, 1953) or azure B (Flax and Himes, 1952) yielded semiquantitative information on protein and RNA content. The metabolism of DNA and RNA also was studied by the autoradiographic method, using larvae which had been exposed to P^{32}.

Wide differences in DNA accumulation were found to occur following the formation of puffs in particular chromosomal regions. In one region, the DNA content consistently remained unaltered, while in a neighbouring one an 8–10 fold increase in DNA regularly followed. Moreover, this marked local accumulation was followed by a gradual diminution in the course of subsequent development.

The results also indicate differences between specific puffed regions in capacity to synthesize proteins and RNA. On some of these, basic proteins and RNA increased by more than 30-fold during the formation of the puff, whereas in others, primarily non-basic proteins appeared, but with no cytochemically detectable RNA. Certain puffed regions show a very high incorporation and rapid turnover rate of RNA, whereas in other puffs it is relatively very low.

It can be concluded that (1) the DNA content at a few, particular chromosomal regions is subject to variation, which shows that the DNA-content is not absolutely constant per chromosome set; (2) puffed regions produce substances of different chemical composition and metabolism, which might be considered as primary gene products; and (3) DNA variation occurs independently in different loci during the development, probably reflecting a functional pattern.

STILES, KARL A. Reproduction in a Mongoloid Imbecile. *Zoology Department, Michigan State University, East Lansing, Mich., U.S.A.*

A woman who had been diagnosed as a mongoloid imbecile gave birth to a child that was also diagnosed as a mongoloid. The mother was 19 years old when the daughter was born. The daughter is illegitimate and the father is unknown. The daughter is unquestionably mongoloid; she has a mongoloid facial expression, thickened tongue,

stubby fingers and toes, low rate basal metabolism, dry skin, cephalic index of 85, and dermatoglyphic records, which were analysed by Dr. Norma Ford Walker and shown to be definitely those of a mongoloid. Her I.Q. is 22, and she functions at a low imbecile level.

The mother's facial expression is not so definitely mongoloid as that of the daughter. Furthermore, her dermatoglyphic records, which were also analysed by Dr. Norma Ford Walker, placed her in the overlap area. Therefore, these dermatoglyphic records neither provide evidence for mongolism, nor do they give any proof that she is not a mongoloid.

The mother does have the following combination of mongoloid characteristics: basal metabolism low, skin dry, depressed nasal bridge, lax joint ligaments, thick broad tongue, cephalic index 84, almond-shaped eyes, stumpy hands and feet, and small round ears. Her I.Q., taken in 1952, was reported as 41 on a Stanford-Binet-Form L, and again in 1956 with the same test as 44.

This study supports the conclusion that a mongoloid child was born to a mongoloid mother.

STINSON, HARRY T., JR. Genome-Cytoplasmic Interactions and Species Crosses in Oenothera. *Connecticut Agricultural Experiment Station, New Haven, Conn., U.S.A.*

The circle-forming behavior of certain Oenothera strains makes it relatively easy to transfer the genome of one species into the cytoplasm of another. The procedure used was to cross a geographical race of the complex-heterozygous species *O. parviflora* as female by an alethal, complex-homozygous race of *O. hookeri*. The F_1 complex combination, alpha *parviflora*·^{h}*hookeri*, gives ⊙ 14 at meiosis. On selfing, ^{h}*hookeri* · ^{h}*hookeri* segregates are obtained in *parviflora* cytoplasm, since *parviflora* was female parent in the original cross. The recovered *hookeri* plants are only slightly different from the original *hookeri* parent, probably owing to *parviflora* genes which entered one, or both, of the recovered *hookeri* complexes through crossing-over. The recovered and original *hookeri* are further alike in their breeding behavior when used as male parents, but are markedly different when crossed as female parents by the same tester strains. *O. hookeri* crossed as female by *O. parviflora* and *O. argillicola* gives chlorotic F_1 plants which die as seedlings. In contrast, the recovered *hookeri* in *parviflora* cytoplasm gives fully green plants when crossed by the same parvifloras, and fully green and green-yellow variegated plants when crossed by the argillicolas. The variegated plants result probably from male transmission of *argillicola* plastids which are incompatible with the ^{h}*hookeri* · ^{h}*argillicola* genome. The recovery of ^{h}*argillicola* · ^{h}*argillicola* plants in *parviflora* cytoplasm is also possible by the method outlined above. When now the cross is made between *hookeri* and *argillicola*, both of which possess *parviflora* cytoplasm, all the progeny are fully green; no variegated plants occur. Thus, for the first time in the American Oenothera material it has been possible to get mature green plants with the genome combination ^{h}*hookeri* · ^{h}*argillicola*, by employing the cytoplasm (plastids) of a third species, *O. parviflora*. That the different breeding behavior of the recovered *hookeri* is not due to the presence of alpha *parviflora* genes acquired through crossing-over in F_1 (alpha *parviflora* · ^{h}*hookeri*) is indicated by the fact that ^{h}*hookeri* · ^{h}*hookeri* plants isolated from the same F_1 in *hookeri* cytoplasm breed as the original *hookeri*.

STONE, W. H., and M. R. IRWIN. Blood Types of the Progeny of a Pair of Cattle Twins showing Erythrocyte Mosaicism. *Genetics Department, University of Wisconsin, Madison, Wis., U.S.A.*

The majority of twins in cattle are zoological chimeras in that they show mosaicism of the erythrocytes, and possibly of other tissues as well as the hematopoietic tissues. The report of erythrocyte mosaicism by Owen (Sci. *102*:400) stated that a twin sire had failed to transmit to any of twenty progeny certain of the antigenic factors of his blood, implying that these antigenic factors were present somatically but not germinally. Progeny from each of a pair of twin bulls with erythrocyte mosaicism have been tested for possible effects of the intimate union of the hematopoietic tissues on the germinal and somatic tissues of each twin. Each twin possessed in approximately equal proportions two antigenetically distinct kinds of blood cells. After differential hemolysis tests, the abbreviated blood types of the two kinds of cells were: type I: $QE_1'I'/BGKO_3A'$ C_1X_1 F/F J/- and type II: $QE_1'I'/BI_1$ C_1X_3 F/F J/- L/- Z/- H'/- representing respectively, four and seven of the ten antigenic systems of cattle blood. Seven progeny from testcross matings of one of the twins (genotype of type I) did not contain BI_1, L, Z, or H' which differentiate type II blood from type I. Similarly, 19 progeny of the co-twin (genotype of type II) did not possess the phenotype $BGKO_3A'$ or X_1 which differentiates type I bood from type II. The progeny of each twin sire possessed in expected proportions only those antigenic factors produced by the genes corresponding to the genotype of the sire. Thus, the intimate association, beginning in early embryonic life, of the two kinds of hematopoietic tissues in each twin did not demonstrably affect either the cellular antigens formed by these tissues, or the genes for the cellular antigens transmitted by each twin to its offspring.

STONE, W. H. *See* BEDNEKOFF, A. G., *et al.*

STORMONT, CLYDE. *See* RASMUSEN, BENJAMIN A., *et al.*

STRAUSS, BERNARD S. Effect of the Distribution of Incorporated Sulfur on the Lethal Effect of Incorporated $S^{35}O_4$. *Department of Zoology, Syracuse University, Syracuse, N.Y., U.S.A.*

In order to study the effect of incorporated S^{35} under conditions of defined radiation dose $S^{35}O_4$ was incorporated into conidia of *Neurospora crassa*. Sulfate was incorporated at different specific activities; so the amount of sulfur incorporated into cysteine or methionine varied. A conidium containing enough S^{35} to give one d.p.m. accumulates a radiation dose from the sulfur of about 400 r per day.

A suspension of 10^7 spores/ml. will take up all the sulfur from a medium 1.8×10^{-5} molar with respect to sulfate in two hours incubation with aeration of 30°. The conidia increase their sulfur content by about 40% and increase in total nucleic acid and average number of nuclei per conidium. Addition of 3×10^{-2} micro mole of methionine per ml. practically prevents incorporation of S^{35} into methionine, but

does not appreciably affect incorporation into protein cysteine. The radiation dose accumulated during the incorporation period is negligible.

Spores are washed, frozen and stored at —10° in small tubes. Frozen conidia not containing radioisotope die off on storage in the cold. The rate of death depends on the amount of sulfur (i.e., of germination) in the limiting incubation medium. After correcting for this difference in physiological state we have failed to demonstrate any differential effect on viability of sulfur distribution (ratio of S^{35} in protein cysteine/methionine) or of sulfur specific activity between 1.8 to 17 millicuries per micro mole. Our best measurements indicate a value for alpha in the inactivation relation $2.3 \log N/N_0 = -\alpha D$ (where D is the total number of disintegrations) of 1.1×10^{-5}. A dose of 73,000 r of chronic internal beta radiation is therefore required to inactivate 50% of a sample of incubated conidia compared to our previously reported 33,000 r for a single X-ray dose to non-incubated conidia. No transmutation effect is evident.

(Work supported under contract AT(30-1) 1138 between Syracuse University and the U.S. Atomic Energy Commission. The assistance of Dr. G. Failla with problems of dosimetry is gratefully acknowledged.)

Strömnaes, Oistein. Mutation Pattern in Two Wild-Type Stocks of *Drosophila melanogaster*. *Institute of Genetics, University of Oslo, Blindern, Norway.*

The mutation pattern has been investigated in two wild-type stocks of *Drosophila melanogaster*, one is Oslo, the other is Iso-Amherst. Males, 1 to 4 hours old, from the 2 stocks were treated with 1,800 r of X-rays, then mated individually to single females for 24 hours and transferred to new females every 24 hours for a period of 12 days. In testing for dominant lethals virgin hybrid females from a cross between cn bw; e^{11} ♀ × Canton-S ♂ were used; in testing for sex-linked lethals the Muller-5 technique was employed, and in the testing for 2-3 translocations a cn bw; e^{11} stock was used.

The daily brood pattern for dominant lethals for the two stocks shows up as similar curves. However, dominant lethal frequency is higher for Oslo stock than for Iso-Amherst stock in all first broods until the seventh brood where it is the same for both. From the eighth to the twelfth brood the situation is reversed: maximum frequency of dominant lethals for the Oslo stock appears in the sixth brood; for the Iso-Amherst stock in the eighth brood.

Tests for sex-linked lethals and 2-3 translocations were carried out only for sperms coming on the third, sixth, and eighth day after irradiation of the males. The data observed for 2-3 translocations are in good agreement with the data found for dominant lethals. Thus, the Oslo stock exhibits the highest frequency of translocations on the sixth day, while the Iso-Amherst stock has the highest frequency of translocations on the eighth day. The frequency of sex-linked lethals is higher in the Oslo stock than in the Iso-Amherst stock for all broods tested, and the highest frequency is found in the eighth brood for both stocks.

The observed data indicate an over-all higher mutation rate in the Oslo stock than in the Iso-Amherst stock. It seems reasonable to assume that the different mutability of the stocks has been achieved by selection during the evolution of the two stocks in order to make them better adapted to their natural environment.

(I wish to express my gratitude to the Norwegian Research Council for Science and the Humanities for financial support received.)

STROUN, J. H., L. R. STROUN-GUTTIERES, J. ROSSI, et M. STROUN. Modifications de la poule Leghorn traitée par de sang de pintade. *Laboratoire Rossi et Clinique Universitaire de Médecine Interne, Genève, Suisse.*

Les auteurs ont administré du sang de pintade à des poussins Leghorn jusqu'à la période de fécondation. Ils observent chez les Leghorn F_1 diverses modifications de leurs caractères dont ils discutent l'origine.

STROUN, M. *See* STROUN, J. H., *et al.*

STROUN, MAURICE. Hybridation végétative chez les plantes. *Institut Botanique de l'Université de Genève, Genève, Suisse.*

Les recherches poursuivies sur diverses plantes montrent que l'on peut modifier dans la génération traitée et dans la descendance les caractères d'une plante ne vivant en parasite sur une plante étrangère que pendant la période F_0.

STROUN-GOUTTIERES, L. R. *See* STROUN, J. H., *et al.*

STUTZ, HOWARD C. Penetrance-Dominance Relationships of a New Macromutation in Rye. *Botany Department, Brigham Young University, Provo, Utah, U.S.A.*

A mutant gene discovered recently in a strain of Turkish rye modifies a key taxonomic character in that mutant plants have several spikelets at each node of the spike. This mutant gene behaves as a simple recessive gene in crosses with annual strains of rye and as a dominant gene in crosses with perennial *Secale* species.

In segregating populations from hybrids with another strain of Turkish rye, mutant plants showed incomplete penetrance within culms in that only some nodes of the spike bore multiple spikelets. In an F_2 population derived from the hybrid with Merced rye (a spring annual variety) there was incomplete penetrance within culms, between culms, and apparently even between plants so that the mutant gene behaved as a sub-recessive. On some plants only a single node on a single spike of an entire plant expressed the mutant phenotype.

In segregating populations from hybrids with perennial species incomplete penetrance within culms but not between culms or between plants was expressed.

The penetrance and therefore the dominance of the gene thus appear to be less a property of the gene itself than a function of the external environment, as demonstrated by the variation of penetrance within and between culms of the same plant, and of the internal genetic environment as demonstrated by the variation of dominance from sub-recessive to complete dominance in different genetic strains. Furthermore, since the different genetic backgrounds are apparently new genetic environments for this particular gene, the dominance properties are apparently independent of any selective forces which several other workers (e.g., Fisher, 1931; Haldane, 1939; Wright, 1934) have suggested as the basis for the origin of dominance.

SUEOKA, NOBORU. Studies on the Genetics of Tyrosinase in *Neurospora*. *California Institute of Technology, Pasadena, Calif., U.S.A.*

A new variant form of tyrosinase in *Neurospora crassa* was obtained from a strain coming from Singapore. This form is different in both electrophoretic mobility and heat stability from other standard forms in our laboratory. Genetic analyses indicate that these differences are determined by the same locus (T-locus) which determines the other structural forms of tyrosinase in *N. crassa*. A second series of experiments is concerned with a strain which produces little or no tyrosinase or laccase (also a copper-containing phenolase). Crossing experiments previously showed that this inability to produce tyrosinase and laccase is controlled by a locus (designated *np*) independent of the T-locus. Heterokaryons of various combinations with regard to the different forms of tyrosinase and the inability to produce the enzyme have been made. It turns out that in the heterokaryotic state with producing strains, the nucleus carrying *np* now produces tyrosinase. For example, the heterokaryon (*np*, T^L) (+, T^S) produces a mixture of T^L (thermolabile) and T^S (thermostable) tyrosinases. This suggests that the normal allele of the *np* locus has some other function in the enzyme formation than the T-locus. Simultaneous blocking of laccase production supports this idea. Several other non-producing strains have been obtained from natural sources and these are being analyzed at present.

SUKHOV, K. S. Phases in Development of Phytopathogenic Viruses. *Institute of Genetics, Academy of Sciences of the USSR, Moscow, U.S.S.R.*

The developmental cycle of the tobacco mosaic virus within the host organism consists of qualitatively different phases. After completion of the reproduction phase, designated as the vegetative phase of development, the virus enters the resting phase. During the vegetative phase the virus diffuses throughout the tissues of infected plant but is unable to infect new plants through the conventional modes of inoculation, i.e., it lacks contagiosity. During this phase specific inclusions, the amorphous X-bodies, can be found in the cells. The vegetative virus is not precipitated by antiserum produced against the purified preparation of the resting virus. High temperature in the absence of light can prevent passage of the virus to the resting phase without inhibiting its reproduction. The virus blocked in this way transforms into the contagious form within 1½ to 2 hours after exposure of the infected tissues to optimal conditions.

The transformation of the vegetative virus into the resting rod-like form involves a transitory stage. During this stage, the elementary virus particles are represented by corpuscles with a peculiar morphology. In this state, the virus exhibits high but unstable contagiosity. The infectious corpuscles are precipitated by antiserum produced against the purified virus preparation isolated from mosaic leaves. The formation of rod-like particles with stable contagiosity is the terminal phase of the developmental cycle of the virus.

Experimental evidence shows that variability of the virus as caused by high temperature is to be related to the vegetative phase of development. The variability of the virus is directed and adequate to the acting factor.

SULKINOJA, MATTI. *See* VAARAMA, ANTERO.

SULLIVAN, ROBERT L. Sex Limitation of Several Loci in the Housefly. *Department of Entomology, University of Kansas, Lawrence, Kan., U.S.A.*

A strain of houseflies (*Musca domestica* L.) has been found in which the expression of the mutant brown body color is restricted to the female. When tested with a strain obtained from Dr. Milani of Pavia, Italy, in which brown body behaves as an autosomal recessive the following results were obtained. The American brown female crossed to an Italian brown male yields all brown flies in the F_1 and subsequent generations. When the normal male of the American strain is crossed to the Italian brown female the males are normal and the females brown bodied in the F_1 and subsequent generations. When the American brown female is outcrossed to a normal male the brown body color is expressed in one-quarter of the flies of both sexes in the F_2 generation.

The males of this strain when crossed with females of a green eye color strain give a similar result. In the F_2 generation green eye color is expressed only in the females. The brown and green loci have been found to be linked in other tests.

These results may be explained by several hypotheses which involve the attachment of a portion of a normal autosome to the Y-chromosome. This currently is being studied by further crosses and cytological examination.

(This investigation was supported by the Medical Research and Development Division of the Office of the Surgeon General, Department of the Army, Contract No. DA-49-007-MD-738.)

SUMPTION, LAVON J., and WILLIAM E. REMPEL. A Study of Evolution in Animal Breeding. *University of Nebraska, Lincoln, Neb., and University of Minnesota, Minneapolis, Minn., U.S.A.*

Animal breeding involves the study of methods of directing the forces of evolution in the improvement of the quantitative economic traits of farm livestock. Because of long generation intervals, limitations in population size and complicating effects of variable environmental conditions the interpretation of evolutionary change in farm animal populations is difficult. The annual rate of change is often indiscernible. A recent study with swine will illustrate how the forces of evolution are being applied to the general problem of animal improvement.

A new line of swine (Minnesota No. 3) was developed by combining 3 outbred breeds and 5 inbred lines: Gloucestershire Old Spot, Large White, Welsh, "C" Line inbred Poland China, Beltsville No. 2, San Pierre, Minnesota No. 1 and Minnesota No. 2. This was a deliberate attempt to increase the initial level of genetic variability as a means of augmenting effective selection within this population. This line was maintained as a single Mendelian population ranging in size from 220 to 490 animals, the progeny of from 9–12 sires and 37–62 dams per generation. Breeding and management practices were designed to capitalize upon natural selection for aggressive breeding ability among selected sires, maternal ability and early sexual maturity among selected dams.

During 6 generations, the performance of line has increased. There has been a significant positive shift in the frequency distribution of 56 and 140 day weights. No change in fertility or survival was evident. Wide variations in body type, color and other superficial traits were observed, factors that with critical analysis may be useful criteria as markers in measuring quantitative variability in animals.

Although genetic improvement of economic traits is slow the animal breeder can make significant progress in a working lifetime provided that: (1) projects begin with populations characterized by a high level of genetic variation, (2) the rate of inbreeding is low enough to provide for extensive recombination, (3) the population is large enough to generate new gene combinations in numbers, and (4) rigorous selection is practised with definite objectives.

SUOMALAINEN, ESKO. Polyploidy in Parthenogenetic Beetles. *Institute of Genetics, University of Helsinki, Helsinki, Finland.*

Of the 34 parthenogenetic weevil species and races studied cytologically, only one (*Polydrosus mollis* in Finland and Poland) is diploid (22 chromosomes), 23 are triploid (33 or 34 chromosomes), 8 are tetraploid (44 chromosomes) and 2 are pentaploid (55 chromosomes). Several of these weevil species are represented by a bisexual as well as by a parthenogenetic race. The former is always diploid, the latter generally polyploid. In addition to the diploid bisexual race, a weevil species may be represented by a number of parthenogenetic races, differing in their degree of polyploidy. The divergent races of a single weevil species have different distributions in all cases investigated so far. This is especially clear in some of the so-called boreo-alpine species. In the Eastern Alps this dissimilar distribution of the divergent weevil races appears to be definitely correlated with the various glacial conditions in different parts of the Alps. It is noteworthy that the relative proportion of parthenogenetic polyploid forms is considerably higher in Northern Europe (Fennoscandia) than, for instance, in Switzerland and in the Austrian Alps.

The chrysomelid beetle *Adoxus obscurus* is bisexual in America and parthenogenetic in Europe. The latter form of this beetle seems to be triploid.

SUOMALAINEN, HANNU O. T. Chromosomal Evolution in the Neuroptera. *Department of Zoology, The University, Turku, Finland.*

In the Neuroptera (lace-wings), representatives of 10 families have been studied cytologically. In general, the more ancient forms seem to have the highest chromosome numbers (up to $n = 13$). In two families at least, Myrmeleonidae and Hemerobiidae, whole arm transpositions (centric fusions) evidently have been the main cause of the decrease of chromosome number during evolution. Large "modern" genera seem to include mostly species with 6 to 8 pairs of metacentric chromosomes. In the Chrysopidae, another mechanism of chromosomal evolution apparently has set in. While the more primitive species possess 6 or 7 pairs of metacentrics, the more highly specialized ones have several acrocentrics in their set of $n = 6$. As has been shown earlier by the author, these insects have strictly localized distal chiasmata at least in the spermatogenesis. Thus, pericentric inversions have an increased chance of becoming established in the karyotype and probably have been responsible for the conversion of metacentrics into acrocentrics in phylogeny. Consideration of certain taxonomical features of the order Neuroptera gives additional support to the conclusion that genetic recombination is strictly delimited in certain families.

SUSKIND, SIGMUND R. The Role of Suppressor Genes in Controlling Tryptophan Synthetase Activity in *Neurospora crassa*. *The Johns Hopkins University, McCollum-Pratt Institute, Baltimore, Maryland, U.S.A.*

Yanofsky and Bonner have shown that specific suppressor genes are capable of partially restoring tryptophan synthetase activity in certain tryptophan-requiring (*td*) mutants of *Neurospora* lacking enzymatic activity. It was previously found that these suppressed mutant strains, which now form active enzyme, continue to synthesize an enzymatically inactive, antigenically related protein (CRM) characteristic of the unsuppressed mutant. Tryptophan synthetase has been prepared from inactive crude extracts of a temperature-sensitive *td* mutant (td_{24}) by fractionation and removal of an inorganic inhibitor. Such active preparations still contain considerable quantities of CRM in addition to the enzyme. The td_{24} enzyme exhibits high *in vitro* sensitivity to both the inhibitor and to zinc. Inhibitor and zinc sensitivity of partially purified enzyme preparations from the following strains have been compared: td_{24}, td_{24} Su_{24} (td_{24} carrying a specific suppressor gene) and wild type. Enzyme prepared from the suppressed mutant appears to resemble that of the unsuppressed td_{24} mutant, and not wild type. These preliminary results suggest that a suppressor gene may have little to do with the actual formation of the enzyme, but rather, it may serve a regulatory function, controlling inhibitor, cofactor or inducer levels or the extent of *in vivo* enzyme-inhibitor and enzyme-coenzyme complex formation. Such an interpretation may be favored by the recent observation that a pyridoxin mutant of *Neurospora*, grown on limiting pyridoxin, forms a protein antigenically related to tryptophan synthetase. Little if any enzyme activity can be demonstrated in crude extracts tested in the presence or absence of pyridoxal phosphate. The quantity of the inactive protein approximates the level of enzyme found in the wild type. Perhaps in coenzyme deficient cells, the apo-enzyme, instead of combining with coenzyme, reacts with a normal cell component, forming an inactive complex. Suppressor gene action will be discussed in light of these results.

SUTTON, H. ELDON. The Distribution of Haptoglobin Types and Their Genetic Implications. *Department of Human Genetics, University of Michigan, Ann Arbor, Mich., U.S.A.*

The frequencies of the three haptoglobin types have been determined in American whites, American Negroes, African Negroes (Liberia and Ivory Coast), American Indians (Apaches), and Asiatic Indians. The highest frequencies of the Hp^1 gene are found in the Negroes (0.80) and the lowest frequency in the Asiatic Indians (0.18). The frequencies for various tribes of the African group suggest intertribal differences. The possible relationship of these to past tribal history will be discussed. The existence of this balanced polymorphic system raises certain questions of selection favoring the heterozygous individuals.

SUYAMA, V. *See* MUNKRES, K. D.

SUYAMA, YOSHITAKA. *See* WOODWARD, VAL W., *et al.*

SUZUKI, KAN-ICHIRO. *See* TANAKA, YOSHIMARO, *et al.*

SUZUKI, YOSHIKO. *See* RASMUSEN, BENJAMIN A., *et al.*

SVESHNIKOVA, IRENE. The Evolution of the Nucleus in the Genus *Vicia*. *Institute of Plant Physiology, USSR Academy of Sciences, Moscow, U.S.S.R.*

The karyological method applied by us in the systematics of the genus *Vicia* has revealed certain laws governing the evolution of that genus. Within the limits of minor taxonomic units, more precise correlative connections between these units can be discovered by the comparative study of changes in their systematic symptoms and in the morphological peculiarities of their nuclei. Given the existence of characteristic laws governing the translocation of interspecific hybrids, these translocations become indicators of the evolution of the species, thereby differing from the translocation of X-ray mutants. The X-ray mutants with surplus chromosomes obtained by us are also of interest in solving the problem of the phylogenesis of the species, since they help to study the localization of definite features in the chromosomes. Polyploid forms have been found only in perennial forms of *Vicia* reproducing from the root stalks, which points to the somatic origin of polyploids in the genus *Vicia*.

SWAMINATHAN, M. S., and A. T. NATARAJAN. Cytological and Genetic Changes Induced by Vegetable Oils in *Triticum aestivum*. *Indian Agricultural Research Institute, New Delhi, India.*

Following our observation (Curr. Sci. *25*:382–4) that oils extracted from mustard (*Brassica campestris* var. *toria*), groundnut (*Arachis hypogaea*) and castor (*Ricinus communis*) are capable of inducing chromosome breakage in root tip cells of *Allium cepa* and *Triticum monococcum*, we soaked seeds of bread wheat (variety C–591) for 24 hours in each of these oils and planted them in the field. A study of mitosis in root meristems showed the occurrence of chromosome and chromatid breaks at metaphase and spindle abnormalities and "error" bridges presumably arising from sub-chromatid breaks, at the anaphase. Binucleate cells with both synchronised and non-synchronised mitosis were also seen. The presence of numerous fragments over several mitotic cycles suggested that exchange or reunion may be rare. However, at metaphase I of meiosis in microsporocytes, one to two quadrivalents were seen in several plants grown from seeds treated with groundnut, mustard and castor oils, indicating that delayed reunion may occur. Pachytene configurations gave evidence for the presence of deficiencies and duplications in the treated material.

Speltoids occurred among the treated plants both in the year of treatment and in subsequent generations. Two short and stiff strawed plants also occurred during the second generation in plants treated with groundnut oil. The variety C–591 is fully bearded and one plant in the material treated with groundnut oil was completely beardless. This plant was selfed and crossed with control C–591. Besides beardless plants, long-tipped, half-bearded and fully bearded plants also occurred in the progeny of the beardless plant. In the F_1 from C–591 beardless $\times$ bearded, fully

bearded and long-tipped plants occurred in the ratio 1:1. The results are interpreted on the assumption that the original beardless plant should have been a chimaera with the germinal layer which gives rise to the glumes being homozygous for a deletion of the awn-producing genes and the layer giving rise to the micro- and megasporocytes being heterozygous for it. In the absence of dominant inhibitors, awn-producing alleles seem to show partial dominance. Thus, the present use of recessive symbols to designate awn-producing alleles by wheat geneticists appears to be incorrect.

Syrop, Harold W. *See* Hughes, Roscoe D.

Szende, K. An Unusual Mutation Frequency to Pentose Utilization in a Strain of *Ustilago maydis*. *Institute of Genetics, Budapest, Hungary.*

When monosporidial glucose-grown cells are spread on d-xylose agar, most of the cells form colonies, but not in a homogeneous manner as the colonies become visible between the third and fifteenth days. In liquid d-xylose medium the growth begins after a long lag. When washed cells were inoculated in a liquid medium without carbon source, there was a residual growth with a 10^2 size. This residual growth is not limited on agar without carbon source. The cells of the colonies picked from xylose plates and respread on xylose plates again showed no lag heterogeneity, perhaps owing to the selection of non-utilizers of xylose. After several passages on glucose media this xylose utilizing property remained stable. This indicates a mutational origin. A mutation rate higher than 10^{-3} was observed, but the examination of exact values encountered some difficulties because of the large residual growth, the high mutation frequency, and the unlimited growth on agar plates. As the mutational frequency is unusually high, thus making it difficult to obtain conclusive evidence for the discontinuous genic change, we cannot, therefore, exclude the possibility of a heterogeneous activation or inhibition of the formation of the xylose-utilizing enzyme complex resulting in a continuous change of the phenotypic expression. In the preliminary experiments the xylose isomerase activity was demonstrated in xylose adapted cells. After passage on glucose the cells lost their enzyme activity. It is possible that following the mutational event the presence of the xylose as a specific inductor is also necessary for the expression of the potency for formation of the xylose-utilizing enzyme complex.

Tabaczynski, Maciej. *See* Kunicki-Goldfinger, Wladyslaw.

Takahashi, Toshiaki. *See* Ikeda, Yonosuke.

Takasaki, Tsuneo. *See* Tanaka, Yoshimaro, *et al.*

TANAKA, KATUMI, and KOJI OHKURA. Evidence for Genetic Effects of Radiation in Offspring of Radiological Technicians. *Department of Human Genetics, Tokyo Medical and Dental University, Tokyo, Japan.*

In an attempt to study the genetic effects of radiation on man, from a list of about 5,000 radiological technicians registered with the Japanese Society of Radiological Technicians, 326 male technicians who had worked for 25 years or more in this field were selected for a study of reproductive history. A questionnaire was sent to all individuals and 750 male pharmacists who were used as controls. Both groups are very similar in economic, social, and educational levels in Japan. Answers were received from 255 technicians (78.2%) and 421 pharmacists (56.1%). However, only 232 of the questionnaires returned by technicians and 300 of those returned by pharmacists were complete and could be used in this study. The average age of technicians (53 years) was greater than that of pharmacists (48 years), but the average age when children were born was very similar (33.8 and 33.1 years). The mean year of birth for the offspring of technicians was 1937 and for those of pharmacists, 1943.

The frequency of sterility was significantly greater for marriages involving radiological technicians (13.80%) than those involving pharmacists (6.00%). The sex ratio of the offspring of technicians (55.25) and of pharmacists (52.59) are both higher than the average in Japan (51.24), but the difference between both groups is also significant ($p = .05–.02$). Frequencies of spontaneous stillbirth and abortion were found to be 6.31% in technicians and 3.54% in pharmacists. Difference between both groups seems to be significant ($p = .05–.02$) while both frequencies are close to the average in Japan. It should be kept in mind that the information on stillbirth and abortion may be incomplete because the events concerned happened about 15 years ago.

Average doses of irradiation are roughly estimated as at least 35 r per year, but the estimate is rather low.

TANAKA, M. *See* KIHARA, H.

TANAKA, NOBUNORI. Cytological Effect of Radioactive Rainfall. *Botanical Institute, Faculty of Science, University of Tokyo, Tokyo, Japan.*

Frequencies of chromosomal aberrations observed in mitotic cells (RTC) of *Tradescantia paludosa* of two experimental lots, one kept under glass and the other left in the open air, were studied in parallel. Experiment has been carried out solely in the rainy season which in Japan lasts about one month, usually around June. Temperature in this season is generally about 20°C.

Radioactivity of the rain was estimated by the radioactivity reduced to the value at 6 hours and 72 hours after the rainfall began, by extrapolation of the decay curve calculated on a sample collected at a fixed time daily and on that taken from the fixed-volume collection of rain water. Radioactivity of falling dusts in the air measured by means of the basin method has been proved to be negligible in the rainy season in Tokyo. Background radioactivity in the air in Tokyo usually stands around 25 cpm.

It seems very likely that differences in the frequencies of the chromosomal aberrations between the two lots are statistically significant, and that the frequency of

aberration increases linearly as cpm/m^2 increases. Accumulated maximum dose of radioactivity for 26 days' exposure was 1.7×10^5 cpm/m^2, and regression equation resulted in $Y = 6.73 + 0.67X$, where X is 10^3 cpm/m^2 and Y, the frequency of aberrations per 10^4 cells.

Daily measurement of radioactivity of rain has been carried out by Drs. Takii, Kadokawa and Fukuda of the Central Meteorological Observatory, Tokyo, to whom thanks are due.

TANAKA, YOSHIMARO, KAN-ICHIRO SUZUKI, and TSUNEO TAKASAKI. Unlimited Progress by Selection in Inbred Lines of the Silkworm. *National Institute of Genetics, Misima, Sizuoka-ken,* and *Kumamoto Branch, Sericultural Experiment Station, Kumamoto, Japan.*

A European, a Chinese, and several Japanese races of the domestic silkworm, *Bombyx mori*, without crossing with other races or strains, were submitted to selection to increase cocoon-shell (silken layer) weight and its percentage to the total cocoon weight, two of the most important characters in sericulture.

The European race, *Giallo Ascoli*, and the Chinese race, *Giallo Oro Chinese*, both univoltines, were imported to Japan from Italy in 1926, and have remained under our control since 1927. The Japanese races were all bivoltines. The univoltines were raised once every year, in spring, the best season for silkworm culture; the bivoltines were reared twice a year, in spring and in summer.

Combined family and individual selections were carried out. For reproduction, inter-family matings were usually preferred to sib matings. Selection experiments on *Giallo Ascoli*, or 010 and *Giallo Oro* Chinese or 610 are still in progress, both attaining the thirtieth generation of inbred selection. The results are unexpectedly far-reaching. In 010, the single cocoon-shell weight which was around 38 cg. in the initial stock advanced to 88 cg. in 1936, 89 cg. in 1953, 90 cg. in 1955, and 97 cg. in 1956. These weights are, so far as I am aware, the highest ever achieved, and there is no sign of stopping the increase as yet. The percentage of cocoon-shell weight to the total cocoon weight has also strikingly gone up during the experiments. The size of larva and cocoon increased markedly, the texture of cocoon surface turned rough, and many other differences from the original stock were observed. No one would now recognize *Giallo Ascoli* in 010, although the latter was raised from the former only by inbreeding and selection. Similar results have been obtained with the Chinese race 610.

Theoretical analysis of these results is not easy. However, the authors are inclined to consider that instability or mutability of genes is concerned with the development of the characters investigated.

Selection in Japanese bivoltines proved, on the other hand, ineffective, partly owing to the fact that mortality in summer culture is usually much higher than in spring culture, thus discarding élite homozygotes which survived the spring culture, and leaving heterozygotes only for reproduction.

TANTAWY, ABDELAZIM OSMAN. Selection Limits with Sib-matings in *Drosophila melanogaster*. *Faculty of Agriculture, University of Alexandria, Egypt.*

Drosophila melanogaster were inbred by brother $\times$ sister mating to almost 100% coefficient of inbreeding. Selection for long or short wing length is effective to the

fourth generation of selection (59.4% coefficient of inbreeding) in the high lines which show 1.0–1.5% deviation from the controls. Selection in the low line is more effective and continues until the fifth generation (67.2% inbred), when these lines show 5.0–6.0% deviation from the control level. Response to selection is ineffective at higher levels of inbreeding in both lines.

Phenotypic variation has been reduced by inbreeding and selection; short wing lines are less variable than long wing ones. The coefficients of variability decline in the earlier generations of selection and then stabilize. Selection was relaxed at 25%, 78%, 88%, and 99% of inbreeding, and the results indicate that relaxed selection, at lower levels, causes the variation to increase to the same level as in the controls, but at higher levels of inbreeding the relaxation of selection has no effect on the variability of the selected lines.

Heritability of wing length was calculated at the previously given coefficients of inbreeding before and after relaxation of selection. Intensive systems of inbreeding before and after relaxation of selection have no effect on the genetic variability at the lower levels of inbreeding (25%), but at higher levels of inbreeding the heritability estimates decline to almost the theoretical expectation. When selection was relaxed for five successive generations, the heritability estimates remained almost constant for the higher levels of inbreeding.

Much improvement for the character concerned is possible under selection with sib-matings before one would expect to approach homozygosity for the specific combining ability genes for wing length.

TANTAWY, A. O. *See* MALLAH, G. S.

TARNASKI, I. *See* PRIADCENU, AL., *et al.*

TASHIAN, RICHARD E. The Role of Enzyme Adaptation in the Heritability of Variation in D-phenylalanine Utilization in Man. *Department of Human Genetics, University of Michigan, Ann Arbor, Mich., U.S.A.*

There is evidence that the normal variation in the ability to utilize the D-isomer of phenylalanine may be to some extent under genetic control. If the availability of D-amino acid oxidase is the limiting factor underlying this variation, then it is essential that the normal variation in this metabolic step be established. The urinary excretion of D-phenylalanine and its corresponding alpha-keto acid analog (phenylpyruvate) after the oral administration of D-phenylalanine were used as a measure of D-isomer utilization and enzyme induction. Repeated administration of the D-phenylalanine substrate resulted, in some cases, in an increased conversion of the amino acid to the keto acid. This apparent induction of D-amino acid oxidase in the kidney and the possible heritability of this variation in phenotypic adaptation will be discussed.

TAUBENECK, UDO. *See* BÖHME, HELMUT.

TAVCAR, A. Genetics and Cytogenetics of Maize with Multiple Number of Leaves and Ears on Stalk Knots and Branches on Tassel Knots. *Institute of Plant-Breeding and Genetics, Faculty of Agriculture, Zagreb, Yugoslavia.*

In the year 1927 in the F_2 generation of a cross of two normal genotypes, one plant of maize developed with leaves on each stalk knot in a decussate position and ears on the middle stalk knots, as well as branches on each tassel knot. By selfing over a period of many years, some plants were raised with the following number of leaves on each stalk knot, ears on the middle stalk knots and branches on each tassel knot: (*a*) 1 (normal), (*b*) 2 (decussate), (*c*) 3 (triple), (*d*) 4 (quadruple) and (*e*) 6 (six). Beside these abnormal plants some others have developed on which the number of leaves on knots, and in connection with them also the number of ears on middle stalk knots and branches on tassel knots, are as follows (the first figure indicates the number of leaves on top knots of the plant, the others give the number of leaves on knots below): (*f*) 1:2, (*g*) 2:1, (*h*) 1:2:1, (*i*) 2:3:1, (*k*) 2:3, (*l*) 2:4, (*m*) 6:3, (*n*) 3:6 and (*o*) 1:2:3:1. On some plants with 2,3,4, and 6 leaves on each stalk knot, the tillers have had normal position of the mentioned organs. On such plants, genetic and chromosomic differences have been observed. With the increase in the number of the leaves per stalk knot, there has been a proportional decrease in the height of the plants and the length and breadth of the leaves. Through backcross of the abnormal plants with 2,3,4 and 6 leaves on each stalk knot to normals it could be shown that the dominance occurs in the following order: normal — 1 leaf (In) $\rightarrow$ decussate $\rightarrow$ 2 leaves (In^d) $\rightarrow$ 3 leaves (In^t) $\rightarrow$ 4 leaves (In^q) $\rightarrow$ 6 leaves (In^s). The genes for the mentioned character are multiple allelomorphic. The number of the leaves on each stalk knot, the number of ears on the middle stalk knots, and the number of branches on each tassel knot are in correlation.

The cytologic investigations have been made on sporocytes in different stages of meiosis. The normal plant, from which the abnormals have been raised, has had $n = 10$ chromosomes. In the abnormal plants the number and the structure of the chromosomes are different from the normal plants. The nearly homozygous abnormal plants have $n = 12$–14 chromosomes (11–12 bivalents and 3–2 univalents).

In meiosis of the abnormal plants many disturbances appeared in the form of bridges, translocations, splittings of chromosomes, etc.

TAYLOR, J. H. *See* WOODS, PHILIP S.

TAYLOR, LEWIS W. Further Studies on Diplopodia IV: Breeding Performance of a Presumably Homozygous Diplopod Male. *Department of Poultry Husbandry, University of California, Berkeley, Calif., U.S.A.*

Diplopodia in White Leghorn chickens is a simple recessive, almost always lethal character (Taylor and Gunns, J. Hered. *38*:66). In fifteen years, only one female and two males exhibiting weak expressions of diplopodia have been reared to maturity. The female laid two shell-less eggs before death; one male produced no semen, the other (P122) was mated by artificial insemination to two lines of diplopod carrier females significantly differing in phenotypic segregation ratios (Taylor, Abbott and Gunns; Abbott, J. Genetics, in press).

With dams from the line segregating in a 3:1 ratio, P122 produced 22 normal :32

diplopod embryos (59.3% diplopods). With the line usually producing less than 6% diplopods, he sired 67 normal :16 diplopod embryos (19.4%). Of 25 normal offspring hatched from these matings, 20 were reared to maturity. One male died before mating and one female was infertile; the remaining 9 males and 9 females all proved in test matings to be heterozygous carriers of the diplopod gene. P122, therefore, meets the criteria for homozygosity of the diplopod gene.

Diplopodia was expressed in P122 by the presence of a single supernumerary toe on one foot. That such an attenuated expression might represent high penetrance of the gene in heterozygous state finds no support from the above data. Furthermore, phenotypic segregation ratios from this male depended on the line of origin of his mates. Additional confirmation of the importance of the genetic constitution for genes other than the diplopod gene in modifying the incidence of diplopodia is derived from these matings. The superior viability and reproductive performance of this diplopod male may be related to the fact that he originated in the third generation following an outcross of two diplopod lines to a non-related inbred strain of White Leghorns.

TAYLOR, ST. CLAIR S. A Linear Growth Process in Twin Cattle. *A.R.C. Animal Breeding Research Organisation, Edinburgh, Scotland.*

Experimental herds of monozygous and dizygous twin cattle belonging to the Animal Breeding Research Organisation have provided a slow motion picture, so to speak, of growth and development.

The rates of emergence of differences in size between twin and co-twin have been studied. A comparison of these rates in the two types of twin indicates the pattern of emergence of genetic differences.

Complementary to rates of emergence of differences in size are their persistence or degree of permanence. Results on that aspect of variation are interpreted in terms of the relative efficiency of selection for growth at various ages. For example, of the variation present between dizygous twins at 2 years of age about one-third appeared between the ages of 9 and 12 months; whereas monozygous twins showed no corresponding increase in variation. Since these dizygous differences emerging between 9 and 12 months of age also show a high degree of permanence, the superiority in mature size, say, of animals selected for large size at 12 as opposed to 9 months of age would appear to be considerable.

A stochastic representation of the growth of linear body measurements is put forward in an attempt to give an integrated description of the emergence and permanence of size differences. The proposed linear growth process is $Y_t = pY_t - 1 + M(1 - p) + E_t$, where Y_t is the size at age t, M is mature size, p is a constant growth potential, and E_t is a growth error, i.e., a deviation from expected growth brought about by illness, say, or some change in feeding. This process takes into account, for example, the broad fact that with respect to the rate of maturing in liberally fed animals, each linear body measurement occupies a fixed position relative to every other throughout life.

TAZIMA, YATARÔ. Mutation Response Pattern of Silkworm Germ Cells to X-Rays. *National Institute of Genetics, Misima, Japan.*

A marked difference has been revealed in the sensitivity and mutability of the spermatogenic cells of *Drosophila* and mouse to X-rays in wide varieties of develop-

mental stages. In both of these animals germ cells in different phases exist together in the same testis and the response pattern was determined by testing successive broods at a definite interval after irradiation. It may, however, be difficult to tell exactly which type of spermatogenic cells are being tested when the adult males have been irradiated. The use of silkworms has an advantage for analysing the problem more precisely because most of the germ cells develop almost synchronously with the development of the parents in both male and female.

X-irradiation was administered to male and female germ cells of the wide-type silkworm at definite stages from fourth larval instar to post pupa at 250, 500, 1,000, 2,000, 3,000 and 4,000 r and mating was made to non-irradiated double recessive *pe re* partners. Response patterns were determined with respect to visible mutation rates at marked loci and dominant lethal mutation rates. In treated males, visible mutation rates showed their peak at the sixth day of stadium 5 (larva), i.e., mostly at the stage of spermatids, and were less in earlier and later stages; while incidence of dominant lethals was highest at the second day of stadium 5, approximately at the spermatocyte stage, which coincided with the stage of marked damage in germ cells.

The irradiated female germ cells showed nearly the same mutation rates at relatively low levels throughout varieties of stages of oogenesis before mid-pupal stage, but after this stage a sudden increase was observable in the mutation rate to the same level as the peak in the male germ cells.

The induced visible mutation rates per r were several times higher in spermatogonia and ten times higher in mature sperms than those of *Drosophila.*

Teodoreanu, Nicolae. L'ophthalmie héréditaire des agneaux de race Tzigaia.
Académie de la République Populaire Roumaine, Bucarest, Roumanie.

En 1945, on a signalé la présence de trois agneaux atteints d'ophthalmie dans l'élévage de "tzigaia bucalai" de Slobozie, Roumanie. Ils ont été sacrifiés. Chaque année, on signalait deux à quatre agneaux atteints d'ophthalmie. A partir de 1951, ce nombre a diminué par suite de l'élimination de la reproduction des parents respectifs. En 1949 et 1950, on a entrepris une étude sur sept de ces agneaux atteints d'ophthalmie. Ces agneaux à la naissance ont un aspect et un poids normaux, ils présentent une grande tache blanche sur le front et la nuque et parfois le bout de la queue est blanc; ils sont vifs, rarement languissants. Les agneaux tzigaia typiques ont la tête et l'extrémité des membres noires, le bout de la queue gris. Deux ou trois jours après leur naissance et parfois même le premier jour, les symptômes de l'ophthalmie apparaissent: conjonctivite, infiltration sanguine diffuse de la cornée, kératite, ulcères sur la cornée, photophobie, cataracte, écoulement de l'humeur aqueuse. L'œil est perdu en deux à trois jours. Les agneaux perdent leur vivacité et leur appétit; à l'âge de deux à trois semaines apparaissent les troubles digestifs (diarrhée), pulmonaires (congestions, broncho-pneumonies), nerveux (ils tournent en cercle). La peau est sèche, la laine tombe, il y a eczéma et le bord des oreilles est enflé. La température oscille, atteint 41° C. La respiration est irrégulière, tantôt accélérée, tantôt rare, profonde ou superficielle, dyspnéique.

Le traitement avec les vitamines A et D, avec la pénicilline, etc., arrête l'évolution des phénomènes sans toutefois les guérir. Dès l'arrêt des injections de vitamines, les phénomènes oculaires et autres réapparaissent. On a réussi à prolonger la vie d'un agneau mâle durant 18 mois; la spermatogénèse manquait. Les agneaux malades

provenaient de parents reproduits par accouplement parent × enfant et consanguinité étroite, méthode qui a servi à la formation de l'élevage de Tzigaia à partir du bélier L_3.

L'ophthalmie des agneaux est de nature héréditaire étant due à un gène semi-létal récessif probablement nouveau et lié au gène, dont le siège est dans le chromosome responsable de la tache blanche. Les agneaux à gène semi-létal à l'état homozygote se reconnaissent à la tache blanche. Ils meurent à l'âge de $2\frac{1}{2}$–$3\frac{1}{2}$ mois. Le gène a un effet pleitropique, primaire sur l'œil et secondaire sur l'appareil digestif, respiratoire, etc. L'ophthalmie héréditaire a probablement apparu sous forme de mutation.

THEILER, KARL. Notochord and Morphogenesis of Hereditary Malformations in Mice. *Department of Anatomy, University of Zürich, Zürich, Switzerland.*

Previous investigations (Theiler, 1950; Watterson, 1954; Holtzer *et al.*, 1955; Strudel, 1955) have shown the notochord to favour cartilage formation of vertebral bodies and differentiation of interverterbral discs in amphibia and birds. Similar experiments in mammals being extremely difficult to conduct examination of mutant mice has yielded rich material for the analysis of the effects of a "genetic notochordectomy" at different stages of development and at different levels. The recessive mutation "truncate" (obtained from M. Dickie, Bar Harbor) suppresses notochord formation in the sacral and/or caudal region from the very beginning, with the same consequences as notochord deficiency in amphibia: namely, fusion of myotomes across the midline, "Basalmassentypus" of neural tube, malformation or lack of vertebrae. The well-known mutations of the brachyury and tailless have been described to affect the notochord (Chesley). The shortness or absence of the tail seems to be due to the absence of its posterior part. The mutation "Short Danforth" (obtained from S. Waelsch) does not suppress notochord formation from the very beginning, but leads to a premature breakdown over its whole length. It seems that the dorso-ventral flattening of all vertebral bodies may be a consequence of this defect, as well as the typical luxation of the axis found in practically all *Sd*-animals. From these three mutants, it can be judged that the notochord may be an important structure in the formation of a normal vertebral column.

THERMAN, EEVA. The Ineffectiveness of Indole-3-acetic Acid on the Meiosis of *Eremurus*. *Botanical Institute, Helsinki, Finland.*

Indole-3-acetic acid is obviously one of the important factors in mitosis, as shown, for instance, in tissue cultures and the root tips of various plants. Nothing, however, is known concerning its possible role in meiosis or its effects on this process. To elucidate this point, a series of experiments was made with *Eremurus himalaicus* as the experimental plant. The experiments were carried out by putting cut inflorescences in the solution to be tested or by halving the spikes and floating them directly on the solution. The highest concentration of indole-3-acetic acid used was 50 p.p.m. and the longest time of exposure 5 days. This treatment did not seem to have the slightest effect on the course of meiosis, which was checked in preparations made every day during the treatment. The various possible explanations for this ineffectiveness are discussed.

THIEME, F. P. *See* BUETTNER-JANUSCH, JOHN, *et al.*

THODAY, J. M. Effects of Disruptive Selection. *Department of Genetics, Sheffield University, Sheffield, England.*

Stabilising selection is here defined as selection which favours the same (mean) phenotype in every generation. Disruptive selection is defined as selection which picks out both extreme phenotypes in every generation. A population of *Drosophila melanogaster* has been exposed to disruptive selection by mating opposite extremes for 40 generations (disruptive selection with negative assortative mating). Another has been exposed to disruptive selection for 31 generations by mating like extremes together and selecting both extremes from the offspring (disruptive selection with positive assortative mating). A third has been exposed to 30 generations of stabilising selection. All were derived from the same wild-stock. Character sternopleural chaeta-number, 4 single-pair cultures are used each generation. Heritability has been tested in these populations by taking out high and low directional selection lines and recording the divergence of their mean chaeta-numbers. Divergence is greatest in the disruptive selection lines, least in the stabilised lines, and (at least in the first generation) intermediate in the foundation wild-stock. It therefore seems that disruptive selection may increase and stabilising selection decrease the effective variety of chromosomes in a Mendelian population. Disruptive selection may therefore be capable of producing polymorphism as Mather has suggested.

The line exposed to disruptive selection with positive assortative mating can be regarded as consisting of two sub-populations, one selected for high and the other for low chaeta-number. Each generation is set up by crossmating the two so that there is maximal (50%) gene-flow between the two. Despite this the two have diverged, showing that isolation is not a prerequisite of such divergence. Random mating involves only 25% gene-flow. Disruptive selection should make possible considerable divergence with 25% gene-flow.

Populations with 25% gene-flow are being maintained and may have progressed sufficiently for report at the Congress.

THOMPSON, MARGARET W. Twin Studies in Cerebral Palsy. *University of Alberta, Edmonton, Alta., Canada.*

Twins are three to four times as common among patients of the Edmonton and Calgary Cerebral Palsy Clinics as in the general Alberta population. The high susceptibility of twins to cerebral palsy is well recognized, and the particular difficulties of a twin pregnancy and parturition are considered to be causal factors in cerebral palsy. However, an analysis of zygosity and concordance in our series shows that monozygotic twins may exhibit concordance for certain types of cerebral palsy, but not for other types. No concordant dizygotic pairs have been observed. The significance of these findings in regard to the etiology of cerebral palsy will be discussed.

THOMSON, L. *See* FOSTER, M.

TING, Y. C., Inversions and Other Characteristics of Teosinte Chromosomes. *Harvard University, Botanical Museum, Cambridge, Mass., U.S.A.*

Three teosinte varieties, Huixta from northern Guatemala, Durango and Nobogame from Mexico, were crossed with two selected inbred strains of maize and cytological studies were made of the F_1 hybrids.

No inversions were found in the F_1 hybrids of Huixta teosinte with maize. This teosinte, however, differs from other previously studied Guatemalan teosintes in having the majority of its knobs located internally. At diakinesis, among 585 microsporocytes examined, 12.3% had two univalents, 0.8%, four univalents.

F_1 hybrids of maize and Durango teosinte proved to be heterozygous for two practically terminal inversions located on the short arms of chromosomes 8 and 9. The inversions include 59% and 56% respectively of the short arms of these chromosomes. At both anaphase 1 and anaphase 2, dicentric bridges and acentric fragments were found. At diakinesis, among 514 cells examined, 0.8% had two univalents. More than two were never observed. Two previously unreported knob positions were found in Durango teosinte: one on the long arm of chromosome 8, and the other on the long arm of chromosome 9.

F_1 hybrids of maize and Nobogame teosinte were heterozygous for 3 inversions, 2 of which are practically terminal and are located on the short arms of chromosomes 8 and 9. The *In 8* represents 62% of the short arm, while the *In 9* represents 59% of the short arm. The third inversion, a paracentric one, is located on the long arm of chromosome 5. The length of the inverted segment is equal to about one-half of the long arm of this chromosome. Both dicentric bridges and acentric fragments were found at anaphase 1 and anaphase 2. Among 514 cells studied at diakinesis, 11.8% had two univalents, 0.4%, 4 univalents. The chromosomes of Nobogame teosinte have only a few knobs: a large terminal knob on the short arm of chromosome 7 and small to medium knobs on chromosomes 1, 2, 4 and 6.

TOKUNAGA, CHIYOKO. The Y Chromosome in Sex Determination of *Aphiochaeta xanthina* Speiser. *Zoology Department, University of California, Berkeley, Calif., U.S.A.*

Certain substrains of *Aphiochaeta xanthina* Speiser ($2n = 6$), belonging to Phoridae (Diptera), derived from strains collected in Okinawa, Japan, show peculiar genetic phenomena. When a male of the substrains is crossed to *brown* or *Delta* female (both third chromosome genes in the original strains), these mutants behave as if they are partially sex-linked in the following generations. On the other hand, when an *Abrupt* or *occhi chiari* female (both are partially sex-linked genes on the X and Y chromosomes) is crossed to the male of the substrains, the *Abrupt* or *occhi chiari* characters segregate as if they are autosomal. These new types of genetic behavior were first found in the males of two wild strains which had reverted from the original *short arista* mutant stock from Okinawa, and later in the male of the *occhi chiari* mutant stock of a Neapolitan strain.

As a possible explanation of the phenomena, the author proposes a working hypothesis involving a Y-linked male determining factor as follows. In this species, there is a strong male determining factor in the normal Y chromosome, and the male sex is determined by the presence of this factor. At times this factor translocates to the third chromosome so that the "special" male which arose now has a male

determining III-Y chromosome, instead of a normal Y chromosome. By crossing over between a normal third chromosome and the III-Y chromosome in the special male, the genes which were on the normal third chromosome become linked with the male factor on the III-Y chromosome and show male-limited inheritance. Data concerning the role of the X chromosome in sex determination have not yet been obtained.

TOWNER, J. W. Amphiploidy in the Cultivated Marigolds (*Tagetes*). *University of California, Los Angeles, Calif., U.S.A.*

A genetic, cytological and morphological analysis of the three cultivated marigold species revealed that *Tagetes patula* L. ($2n = 48$) is a typical genomic allotetraploid which probably originated in Mexico by hybridization between *T. erecta* L. ($2n = 24$) and *T. tenuifolia* Cav. ($2n = 24$), or species closely related to them, and became established as a fertile species by chromosome doubling. The three species were fully fertile and normal in meiosis with no evidence of multivalents. Hybrids between the putative parent species *T. erecta* × *T. tenuifolia* were difficult to produce, very weak, almost completely sterile, and averaged only 2.4 bivalents per PMC. The sterile triploid ($2n = 36$) hybrids *T. patula* × *T. erecta* and *T. patula* × *T. tenuifolia* usually had 12 bivalents and 12 univalents at MI. The corresponding hexaploids ($2n = 72$) were only partially fertile, frequently had the maximum expected pairing of 12 quadrivalents plus 12 bivalents at MI, and showed complex segregations when self-fertilized. A fertile, true breeding, synthetic allotetraploid, *T. erecta-tenuifolia*, was produced by doubling the chromosome number (with colchicine) of the corresponding sterile diploid hybrid. This synthetic allotetraploid was morphologically, cytologically and genetically very similar to the natural allotetraploid *Tagetes patula*. Hybrids between these two allotetraploids were easily produced, highly fertile, and had 24 bivalents in 80% of MI (no multivalents). F_2 segregations of these hybrids demonstrated that *T. patula* and *T. erecta-tenuifolia* held in common the independent gene loci *sg* (flower doubleness), *lt* (short-day flowering), *a* (ligule anthocyanin), and *ai* (ligule anthocyanin inhibitor). Thus the behavior of the various hybrids indicated that the structurally distinct genomes of *T. erecta* and *T. tenuifolia* are very similar to the corresponding subgenomes of *T. patula*.

TOWNSEND, J. IVES. *See* CORDEIRO, A. R., *et al.*

TRASLER, DAPHNE G., and F. C. FRASER. Factors Underlying Strain, Reciprocal Cross, and Maternal Weight Differences in Embryo Susceptibility to Cortisone-Induced Cleft Palate in Mice. *McGill University, Montreal, Canada.*

The developmental age (as measured by external morphological criteria) at which embryonic palate closure occurs in mice was found to differ in the A/Jax and C57BL strains of mice, the reciprocal crosses, and backcrosses, in the following manner: C57BL ♀ × C57BL ♂ = C57BL ♀ × A/Jax ♂ < A/Jax ♀ × C57BL ♂ < (A/Jax ♀ × C57BL ♂) ♀ × A/Jax ♂ = (C57BL ♀ × C57BL ♂) ♀ ×

A/Jax ♂ < A/Jax ♀ × A/Jax ♂. This series corresponds closely to the order of the susceptibility of these crosses to the cleft palate producing properties of cortisone, such that those crosses with palate closure occurring earlier in development show greater resistance.

The frequency of cortisone-induced cleft palate embryos from "light" mothers has been shown to be significantly higher than from "heavy" mothers (Kalter, J. Exp. Zool. *134*:449). In the present study the palate closure of untreated backcross embryos from "light" and "heavy" mothers was compared. It was found that there was a slight, but significant, tendency for palate closure of embryos from "light" (susceptible) mothers to start later than that of embryos from "heavy" (resistant) mothers.

Among cortisone-treated backcross embryos after the normal time of palate closure, those with cleft palate tended to have lower (p .1–.05) morphological ratings and lower (p .02–.01) weights than embryos from the same litters with palates closed.

The maternally influenced (reciprocal cross and weight) differences in embryonic susceptibility to cortisone-induced cleft palate could be due to differences in maternal handling of the cortisone or to differences in embryo reaction to cortisone. The present studies suggest that the determining factor is the state of readiness of the particular embryo and that this readiness has been predetermined by the maternal physiological state, the maternal genotype, the embryonic genotype and the interaction of these factors.

Tsuda, Seizo. Some Features of Fine Structure of Hyphal Cells of Wild Type of *Neurospora crassa*, as Observed with the Phase and Electron Microscope. *Department of Anatomy, University of Washington, School of Medicine, Seattle, Wash., U.S.A.*

Most of the observations were made on vegetative young mycelia cultured on minimal medium. In living hyphae, phase contrast microscopy reveals nuclei, large bright granules, many mitochondria and minute dense granules. The nuclei are commonly rounded or oval and are frequently observed to move from one cell to another through the center pores of transverse cell walls. The mitochondria appear round or as short rods. All the inclusions inside the cytoplasm show vivid motion and are continuously changing their position. Five or six nuclei can be observed in each cell.

As seen with the electron microscope, the *Neurospora* hyphae are surrounded by a cell wall about 2,300 Å thick. The cell membrane is about 60 Å thick and is very extensively folded, forming many small processes and pits or caveolae close to the inner surface of the cell wall. The surface area of the cell membrane is much greater than that of the cell wall.

Nuclei are surrounded by inner and outer membranes, featured by pores. The diameters of the nuclei are 1.5 to 2 microns.

The profiles of the mitochondria are predominantly ellipsoidal and are 0.2 to 0.3 microns in diameter. They are surrounded by membranes and show inner fine structure similar to mitochondria in animal cells, with a matrix interrupted by flattened cristae. At various sites in the cytoplasm, one may observe a number of extremely dense granules about 2,500 Å in diameter. These appear singly or in clumps and resemble lipid granules in the higher organisms. One can also recognize

endoplasmic reticulum in the cytoplasm. This shows flattened vesicles bounded by membranes.

Thus, the hyphae cells of *Neurospora* have many features of fine structure resembling those described for a number of animal and higher plant cell types. However, with respect to certain details, specialized developments can be recognized.

TURCOTTE, E. *See* BURNHAM, C. R., *et al.*

TURPIN, RAYMOND. *See* LEJEUNE, JERÔME.

UCHIDA, IRENE A. The Genetics of Hypercholesterolemia. *Hospital for Sick Children, Toronto, Canada.*

A large Ontario kindred with essential hypercholesterolemia has been investigated in detail with the purpose of clarifying the genetic mechanism involved. The proband is a severely affected child, the offspring of two unrelated individuals with hypercholesterolemia. The abnormal gene can be traced through several members of both families as a simple autosomal dominant with complete penetrance, the severe condition in the proband representing the homozygous state. This is evidence supporting a theory of incomplete dominance.

Although it has been known for some time now that an abnormally high cholesterol level is an inherited trait, the exact mode of inheritance has in recent years been a controversial subject. It is evident that the argument can be resolved by using a different diagnostic criterion which must take into consideration primarily the cholesterol level at specific ages rather than the mere presence or absence of xanthomata and coronary artery disease. Since cholesterol levels increase with age and since the appearance of xanthomata is even more dependent upon the passage of time, detection of homozygosity should be restricted to children. At the present state of clinical knowledge the homozygote would appear to be sublethal.

Moreover a continuous variable such as serum cholesterol levels raises the problem of defining the limits for the norm. These ill-defined limits, as well as the influence upon lipid metabolism by several abnormal conditions should caution one from accepting a slightly elevated cholesterol level as diagnostic of essential hypercholesterolemia without a suggestive family history.

UHRING, JOSEPH. *See* EMSWELLER, S. L.

ULRICH, HANS. The Mutagenic Action of X-Rays on Uncleaved *Drosophila* Eggs and Its Dependence upon Oxygen. *Zoologisches Institut der Eidg. Technischen Hochschule, Zürich, Switzerland.*

Uncleaved eggs of *Drosophila melanogaster* were X-rayed totally or partially, the nucleus-containing anterior or the non-nucleated posterior halves being treated

separately. Embryonic and postembryonic mortality was registered, and dose-effect curves were constructed. The frequencies of sex-linked recessive lethals were determined by a modified Muller 5-technique.

The shape of the curves together with other criteria suggests that killing of eggs by X-raying them totally or in their anterior halves is due to the induction of dominant lethals in the irradiated nucleus, whereas X-raying of posterior halves kills the egg by damaging the cytoplasm. In all three cases the mortality is greatly reduced if we irradiate in pure nitrogen instead of in air.

Total irradiation with 1,000 or 1,050 r results in 7.27% sex-linked recessive lethals. This surprising high frequency may be due to a higher mutation sensitivity of the zygote or to absence of selective elimination of gametes with induced recessive lethals, which might happen in the common case of the irradiation of adult males. X-raying of anterior halves gives comparable results.

Irradiation of posterior halves (with much higher doses because of their much lower radiosensitivity) induces recessive lethals also. Their frequencies are correlated with the embryonic mortality in the same manner as those of X-raying the total eggs or anterior halves. There are good reasons to believe that the recessive mutations induced in the nonirradiated nucleus by the irradiation of the cytoplasm of the posterior half are due to an indirect mutagenic action rather than to scattered X-rays or secondary electrons which might have reached the shielded nucleus.

Embryonic and postembryonic mortality, as well as frequency of recessive lethals, is greatly reduced if total eggs or anterior halves are X-rayed in pure nitrogen instead of in air. When, however, posterior halves are X-rayed, nitrogen only reduces the embryonic and postembryonic mortality, but apparently not the frequency of recessive lethals.

In conclusion, the method of irradiation of uncleaved eggs of *Drosophila* instead of adult flies (as is usually done) seems to offer some advantages in analysing the mutagenic action of radiation, especially the indirect or the delayed effects, also the dependence of mutagenesis on oxygen and on other chemicals, as well as the analyses of other problems.

UMAERUS, MAGNHILD. *See* GOWEN, JOHN W.

VAARAMA, ANTERO, and MATTI SULKINOJA. On the Origin of Variability in China Aster (*Callistephus chinensis*). *Department of Botany, University of Turku, Turku, Finland.*

Callistephus chinensis, the only species of its genus, introduced to Europe in the year 1728, immediately acquired great popularity as an ornamental. A great number of garden forms differing from each other mainly by flower characteristics have been developed. Since cytogenetic mechanism leading to polymorphism has not been sufficiently studied, 30 types representing all the commonly cultivated garden forms were examined cytologically.

All the types investigated were diploid ($n = 18$). No analysable differences were observed in the karyotypes of fairly symmetrical structure. Two pairs of satellite chromosomes are present, both of them characterized by a segment of heterochromatic material.

Both mitosis and meiosis of the wild type is regular and the pollen good. Among

the garden forms numerous examples of lability in the chromosome mechanism were found. One plant was aneuploid ($n = 19$). A number of plants with small fragment chromosomes were observed. In meiosis numerous cases of multivalent and univalent formation were noticed. Usually pollen formation was also irregular. Many other types of irregularity in the meiotic mechanism were encountered, but most of them were of local occurrence within the flower head and probably are often connected with the development of pleniflory with its tendency to cause sterility.

The China aster seems to be a counterpart of *Lathyrus odorarus* in its development of polymorphism. One rather monotypic diploid species has given origin, mainly by gene mutations and without hybridization and polyploidy, to a wealth of garden forms. Although it was not possible to establish any correlation between cytological deviations and particular phenotypes, the great lability in the chromosome mechanism may also be considered to have contributed to the origin of the variability.

VALENCIA, J. I. *See* VALENCIA, R. M.

VALENCIA, R. M., J. I. VALENCIA, and W. F. KIRSCHBAUM, A Quantitative and Qualitative Analysis of Mutations Induced by Fast Neutrons. *Atomic Energy Commission of Argentina, Buenos Aires, Argentina.*

Drosophila melanogaster males of a stock which has no visible genetic markers but which has, in the X-chromosome, the inversions sc^{S1} In49 sc^{8} (H. J. Muller's "Insc" stock) were irradiated with fast neutrons delivered by a cascade accelerator. The males were collected as virgins and aged 2–3 days before treatment. They were then mated to virgin females of a stock which contains in one X-chromosome y sc^{S1} B In 49 v and in the other a series of recessive markers. The males were carried through five broods, brood A lasting three days, broods B, C, D and E two days each. In each brood they were given fresh virgin females. Thus the offspring of the different broods should represent sperm that was irradiated in successively earlier stages of spermatogenesis. Three doses were given, these being equivalent in effectiveness (in terms of percentage of recessive lethals in the X) to approximately 750 r, 1,500 r and 2,250 r of X-rays. All mutations, visibles and lethals, are being analyzed cytogenetically. A considerable number of mutations have already been collected, but a very large number will be required before conclusions can be drawn. Results will be discussed at the Congress.

VAN DER VEEN, J. H. *See* PRAKKEN, R.

VETUKHIV, M. O., and J. A. BEARDMORE. Effect of Environment upon the Manifestation of Heterosis and Homeostasis in *Drosophila pseudo-obscura. Department of Zoology, Columbia University, New York, N.Y., U.S.A.*

To investigate the thesis that the manifestation of heterosis in hybrid F_1's may be different in different environments, two laboratory populations of *Drosophila pseudo-*

obscura were crossed and P, F_1 and F_2 were compared concurrently in three environments. Larval viability, egg production and wing asymmetry were used as measures of heterosis.

At 25°C we found (*a*) heterosis in larval viability and significantly lower variation (= superior homeostasis) in survival; (*b*) heterosis in egg production; but (*c*) no statistically significant differences in wing asymmetry. At 22°C no heterosis was detected.

At temperatures fluctuating between 17°C and 27°C (mean = 22°C) no heterosis was detected.

There are two possible explanations of these results; both however assume that 25°C is for these flies a relatively stringent and difficult environment. The first involves "special adaptation" related to the fact that the parental populations had been maintained at 15°C for a number of years and are therefore adapted to this temperature. The 22°C and F 17°/27° environments are consequently less difficult (because less distant from 15°) than is the 25° condition. The second explanation rests on "general adaptation": that is, for the species of *D. pseudo-obscura* as a whole 25°C is a less favorable environment than either of the others. At present we cannot tell which of these two possibilities may be correct and more work is needed to elucidate this problem. It seems reasonable to assume, however, that in this experiment 25°C is the most unfavorable condition (larval viability, shortest life, lowest egg production). These results suggest that there may often exist a "threshold" of environmental stringency which must be reached before heterosis is manifested.

VICKERY, ROBERT K., JR., and BARID B. MUKHERJEE. Cytogenetic Studies of the Patterns of Evolution in the *Mimulus guttatus* complex (Scrophulariaceae). *Department of Genetics, University of Utah, Salt Lake City, Utah, U.S.A.*

The relationships of the various members of the *Mimulus guttatus* complex were studied by means of chromosome counts, experimental hybridizations, and the analysis of chromosome pairing in the hybrids. *Mimulus guttatus*, the main species of the complex, has $n = 14$ chromosomes although the tetraploid-like behavior of the chromosomes of two of its races indicate that the base number is $x = 7$. Two other races of *M. guttatus* exhibit irregular meioses resulting in functional microspores with from 13 to 16 chromosomes. Possibly this is the mechanism that produced the aneuploidy frequently observed in the complex.

Mimulus guttatus hybridizes readily with *M. lyratus* ($n = 14$) and with the $n = 14$ races of *M. nasutus*. Their chromosomes pair regularly indicating the presence of a common genome in these three species. Moderate to strong barriers separate *M. guttatus* from the $n = 13$ races of *M. nasutus* and from *M. tilingii* ($n = 14, 15$), *M. glaucescens* ($n = 14$), *M. glabratus* ($n = 14$) and *M. platycalyx* ($n = 15$). The chromosomes of these species show some synaptic irregularities in their hybrids with *M. guttatus*. However, despite the presence of aneuploidy in several cases, the genomes of these five species are still essentially homologous to that of *M. guttatus*.

The same genome is found in the South American species, *M. parviflorus* ($n = 45$) and *M. pilosiusculus* ($n = 46$). F_1 hybrids of these species with *M. guttatus* are sterile, but their chromosomes regularly exhibit 14 bivalents and 31 or 32 univalents in meiosis. The basic genome appears to have evolved appreciably in

North American *M. corallinus* ($n = 24$). The sterile F_1 hybrids of *M. corallinus* × *M. guttatus* exhibit from 2 to 14 bivalents plus from 0 to 4 trivalents. Perhaps *M. corallinus* is an aneuploid derivative of a tetraploid form. Lastly, the genome of the South American *M. luteus* ($n = 30, 31, 32$) appears to be distinct as it is doubtful that any of its chromosomes pair with those of *M. guttatus*.

The *Mimulus guttatus* complex contains at least one and perhaps two distinct genomes. These basic genomes have evolved by numerous gene mutations, frequent aneuploidy, and occasional polyploidy to produce the many polymorphic species and races of the complex.

VITAGLIANO, TADINI G. *See* MONTALENTI, G., *et al.*

VOGEL, FRIEDRICH. Family Investigations on the Genetics of the "Normal" EEG in Man. *Max Planck-Institut für vergleichende Erbbiologie und Erbpathologie, Berlin-Dahlem, Germany.*

By comprehensive investigations of twins it has been shown that the individual character of the EEG under normal conditions is almost exclusively determined by genetic factors. This result encouraged us to begin a more exact genetic analysis on the basis of family studies. We started with two special types of the normal EEG: the moderately frequent low voltage EEG, and the EEG type with predominantly 4–5/sec. α-variants, which is very rare. In both we were able to detect a relatively simple genetic situation, which is discussed in detail. We would like to emphasize, however, that these investigations are to be considered only as the first step into the analysis of a group of traits which ultimately may prove to be genetically very much more complex.

VOGEL, HENRY J. *See* COCITO, CARLO.

VOJTÍSKOVÁ, MARTA. Distant Hybrids from Parents with Reduced Immunological Reactivity. *Czechoslovak Academy of Science, Institute of Biology, Department of Experimental Biology and Genetics, Prague, Czechoslovakia.*

In hybrids between guinea-fowl hen and domestic fowl cock, if they hatch at all, there is usually a high incidence of malformation. Adult hybrids have been described only twice.

We have attempted to determine whether a distant cross of the above combination might not be favourably influenced by a decrease in the reciprocal immunological reactivity of both the parent birds. Present results lend support to the feasibility of this assumption since the fertilization percentage in experimental crossings far exceeded that of the control guinea fowls and since we obtained four hybrids which have survived into maturity (they are now 16–18 months old).

Procedures influencing immunological reactivity of experimental animals were as follows: in guinea-fowl hens long-term administration of fowl blood during

maturity, and in cocks intra-embryonic injections and long-term administration of guinea-fowl blood from birth.

The histological examination of the gonads of four hybrids from experimental crossing, which died during hatching, showed that all were males. The seminiferous tubules were normally developed, but did not contain spermatogonia, so that the hybrids would evidently have been sterile.

The serological analysis showed no "hybrid substance" in our hybrids apart from the common and specific antigens for both parent species. Further experiments are in progress.

VON BORSTEL, R. C. Dominant Lethal Mutations in *Habrobracon* and *Drosophila*. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

In the parasitic wasp *Habrobracon*, unfertilized eggs become normal haploid males; fertilized eggs, diploid females. Three types of dominant lethal mutations have been identified in *Habrobracon*. Type I dominant lethality kills the embryo during the first few cleavages and is believed to represent a defect in deoxyribonucleic acid synthesis. These account for 60–80% of the deaths when eggs or sperm are irradiated and Type I lethality is unaffected in expression by fertilization. Type II kills the embryo after blastula formation and before the embryo hatches if the embryo is haploid, after hatching if the embryo is diploid. These are called conditionally delayed dominant lethal mutations and are characteristic, with Type I, of *Habrobracon* eggs irradiated in the first meiotic metaphase. They are believed to be manifestations of chromosome imbalance. Type III kills the embryo after blastula formation and before hatching whether the embryo is haploid or diploid. Death may occur later in embryogeny in the diploids than in the haploids, but always before hatching. Type III, with Type I, is characteristic of *Habrobracon* eggs irradiated in the first meiotic prophase.

In *Habrobracon*, five translocations, when heterozygous, produced aneuploid embryos that all died under the same circumstances that characterize Type II dominant lethality. Type III dominant lethals are exactly mimicked by aneuploid embryos from triploid *Habrobracon* females, indicating that Type III radiation-induced dominant lethality may be caused by chromosome loss. Type I dominant lethality remains specifically induceable only with mutagenic agents.

In *Drosophila*, dominant lethal analogues of Type I and Type III have been observed following X-irradiation. Type III is mimicked in *Drosophila* by aneuploids from triploid females or translocation heterozygotes; Type I is specifically induced by radiation. Even *Drosophila* zygotes deprived by genetic means of the X and Y chromosomes and lacking the associated nucleoli develop further than Type I dominant lethal embryos.

WAGNER, R. P., and ARLOA BERGQUIST. The Accumulation of Probable Isoleucine and Valine Precursors by a Mutant of *Neurospora*. *Department of Zoology, University of Texas, Austin, Tex., U.S.A.*

A mutant of *Neurospora crassa*, T304, which will grow only in the presence of both isoleucine and valine, accumulates two compounds, acetylethylcarbinol and acetylmethylcarbinol in its growth medium. These compounds have been identified as their

pteridine derivatives after their reaction with 2, 4, 5-triamino-6-hydroxypyrimidine dihydrochloride.

Acetylethylcarbinol and acetylmethylcarbinol can be derived from alpha-aceto-alpha-hydroxybutyric acid and alpha-acetolactic acid, respectively, by decarboxylation. Decarboxylation occurs spontaneously at acid pH, but is also catalyzed by an enzyme in *Neurospora* which is active in the acid range. Other work has shown that alpha-acetolactic acid and alpha-aceto-alpha-hydroxybutyric acid are probably precursors of valine and isoleucine respectively. Hence the demonstration that a mutant requiring valine and isoleucine accumulates the derivatives in an acidic milieu (the mutant will grow only at an acid pH) indicates that these acids are indeed precursors to valine and isoleucine.

The carbinols accumulate in the medium in detectable amounts only when isoleucine and valine are present in sub-optimal amounts for growth. This is probably, therefore, another example of an end product inhibiting its own synthesis.

T304 segregates as a single gene. It is linked to 16117, another isoleucine-valine mutant.

WAGNON, K. A. *See* ROLLINS, W. C.

WAHRMAN, J., and A. ZAHAVI. Cytogenetic Analysis of Mammalian Sibling Species by Means of Hybridization. *Department of Zoology, The Hebrew University, Jerusalem, Israel.*

Three allopatric forms of *Gerbillus pyramidum* (Rodentia : Gerbillinae) are indistinguishable by conventional taxonomic criteria, but differ widely in the form and numbers of their chromosomes: (1) Algeria—$2n\,♂$ 40, metacentric chromosomes (m.c.) 38, acrocentric chromosomes (a.c.) 2, chromosome arms (c.a.) 78; (2) Israel-coastal plain—$2n\,♂$ 52, m.c. 22–24, a.c. 28–30, c.a. 74–76; (3) Israel-Negev—$2n\,♂$ 66, m.c. 10, a.c. 56, c.a. 76; (4) hybrid between (1) and (3) —$2n\,♂$ 53, m.c. 24, a.c. 29, c.a. 77. These data suggest a Robertsonian relationship.

A male hybrid was obtained by mating a female from the Israel-Negev population ($2n = 66$) to a male from Beni-Abbes in Algeria ($2n = 40$). The fertility of the hybrid was not definitely ascertained, but on dissection the testis proved to contain spermatozoa displaying no gross morphological deviations from the normal. Spermatogonial metaphase plates possess 53 elements. Diakineses and first metaphases reveal a considerable regularity in chromosome association: the 15–17 elements comprise 3 bivalents, 9–11 trivalents which result from the pairing of 2 acrocentrics with 1 metacentric chromosome and 3–4 chain configurations each made up of 5–7 chromosomes. A few cells contain a single univalent, which may have become detached from the end of a chain. Second metaphase plates are of many kinds differing in number of chromosomes and chromosome arms.

The analysis of the synapsis in the hybrid thus discloses a high degree of genetic affinity between populations which are some 4,000 km. apart and which differ by as much as 26 chromosomes. The numerous trivalents bear witness to the homology of acrocentrics from the Negev population to metacentrics of the Algerian form. The compound chains suggest that certain metacentrics of the two genomes are

homologous for only one of their arms (Oenothera fashion) owing to independent Robertsonian changes.

The three strains are interpreted as a series of allopatric sibling forms of common ancestry. The main discrepancy between its members is due to organization of the genetic material in different numbers of linkage groups. The evolutionary significance of changes in chromosome numbers between related species are discussed.

WAKONIG, RESA, and J. T. ARNASON. Effects of Triazine on Chromosomes. *Biology Department, University of Saskatchewan, Saskatoon, Sask., Canada.*

When roots are treated with the alkylating agent 2:4:6 tri (ethylene imino)–1:3:5 triazine, the compound is able to penetrate the nucleus and produce conspicuous changes in the chromosomes. Roots of the bean, *Vicia faba*, treated for 12 hours in a 10^{-5} M solution of triazine at a constant temperature of 20°C have few aberrations until about 12 hours after the conclusion of treatment; however, anaphase aberrations become numerous at 12–36 hours with the peak frequency near 24 hours. At the peak nearly 30% of the cells are disturbed. When either the exposure time or the concentration is reduced there is a corresponding reduction in anaphase aberrations.

All roots collected for observations on metaphase chromosomes were prefixed in 0.05% colchicine for two hours. Collections were made at 12-hour intervals up to 72 hours. At the peak period (24 hours) many aberrations occur. These include free fragments (isolocus breaks), and many paired constrictions (incomplete breaks). Chromatid interchanges occur as shown by tri- and quadriradial configurations. Dicentric chromosomes have not been seen. A few intrachanges have been observed, all involving a part of the satellite (largest) chromosome. Sister reunion is more common in acentric than in centric fragments.

Most of the breaks and exchanges, whether involving the satellite chromosome or the short ones, occur in heterochromatic regions. In the satellite chromosome the regions on either side of the centromere are most frequently affected. In quadriradial configurations involving a satellite chromosome the other chromosome is nearly always a short one. Thus chromatid interchanges do not usually involve exchanges at homologous loci.

Barley seed germination is inhibited nearly 100% by a 24-hour treatment with 10^{-3} M triazine. Hexaploid wheat similarly treated has less than one-half of its seedlings stopped. Surviving seedlings are being grown for mutation studies.

WALKER, G. W. R., and R. A. MILLER. Linkage and Cytological Studies of Barley Mutants. *Department of Plant Science, University of Alberta, Edmonton, Alta., Canada.*

A number of mutants in commercial barleys have been investigated to determine the linkage groups involved. Ten of these mutants were produced at the University of Saskatchewan, two at the University of Alberta, and seven were from other sources. These mutants show linkage to known markers (*v, b, trd, n, br, r, s, an*). The following genes have been shown to be allelic: "bracteatum" and "third outer glume," and two glossy-leaf mutations in irradiated Montcalm and chemically treated Gateway. "Peto's mutant" carries the "orange lemma" gene, and two "bushy"

mutants in irradiated Montcalm carry the "elongated outer glume" gene. A highly significant difference in linkage of genes on linkage group VI has been found between populations from the "intermedium" side-florets and central florets of the F_1 of a cross between a glossy-leaf mutant and Nigrinudum I. Translocations have been cytologically identified in the F_1's and F_2's of the testers Nigrinudum I and Brachytic 119 with "longawned outer glume 3" and "early 6" (54/1121 r65 and 54/1121 r95, Gustafsson), "sterile inisshapen heads" and "cholorotic stripe" (L50–305 and L50–321, Lambert) and a "dense head" mutant (L5220, obtained from Dr. E. Larter, University of Saskatchewan).

WALKER, NORMA FORD. Nephrogenic Diabetes Insipidus and Its Occasional Association with Mental Retardation. *University of Toronto and Hospital for Sick Children, Toronto, Ont., Canada.*

During the years from 1952 to 1957 five children affected with nephrogenic diabetes insipidus were admitted to the Hospital for Sick Children in Toronto, Canada. A sixth child was located through an unaffected relative in the routine assembling of a pedigree. Of the six, two are considered to be mutations. The remaining four have extensive pedigrees with other affected members and all four family groups originate from a small area within a radius of 50 to 100 miles. The younger generations who have been interviewed do not know that they are related, but an intensive search concerning ancestors (who were early settlers in Canada) is in progress. Each family group knows only that his own family has the "curse of the water-drinkers." Inherited as a sex-linked recessive, the anomaly is mildly expressed in some carrier women. A serious aspect of the disease is its occasional association with mental retardation.

WALTER, EDWARD. *See* LAUPRECHT, EDWIN.

WALTERS, JAMES L. Translocation Heterozygosity in Natural Populations of *Paeonia brownii*. *Department of Biology, University of California, Santa Barbara College, Goleta, Calif., U.S.A.*

Paeonia brownii and *P. californica*, the only species of this genus native to the Western Hemisphere, closely resemble each other in morphology (they were long thought to comprise a single species) and in cytological behavior, and in the latter respect they differ markedly from the rest of the genus. *P. brownii* ranges over a large portion of the northwestern United States, with much climatic variety, while *P. californica* is restricted to a narrow coastal strip in California. Each species has evolved an extensive system of permanent structural heterozygosity, of which translocations are the most conspicuous feature. With $n = 5$, 7 different pairing types (combinations of pairs and rings of chromosomes) may be formed, and all 7 have been found in each species. Within the ring-forming types, it is possible to recognize a number of different zygotic types (arrangements of chromosomes) because 2 of the 5 pairs are morphologically distinguishable. It was earlier found that *P. californica* shows a predominance of plants with or without translocations (5 pairs of

small rings), with the complete ring of 10 the least frequent type, and that most areas are cytologically heterogeneous, being occupied by 2 or more different pairing or zygotic types. *P. brownii*, in contrast, appears to consist predominantly of plants with large rings, the ring of 10 being the most frequent, and there are seldom more than two types and often only one in a given area. Thus *P. brownii* appears to have evolved farther along the road to extreme translocation heterozygosity than has *P. californica*. This observation is consistent with other evidence suggesting that *P. brownii* is the older of the two species, and with the evidence of its much wider geographic distribution. Preliminary studies indicate further similarities between the two species with respect to other cytological irregularities, including inversions and fragmentation.

WANG, CHI-WU, and T. O. PERRY. The Ecotypic Variation of Dormancy, Chilling Requirement, and Photoperiod Response in *Betula* Species. *School of Forestry, University of Florida, Gainesville, Fla., U.S.A.*

Under uniform growing conditions marked variation in the time of growth initiation and the time of cessation of height growth was observed between birches of different geographic origins. To study the ecotypic variation in *Betula* species, a collection of white birch (*Betula papyrifera*) and yellow birch (*B. alleghaniensis*) was treated with various combinations of winter chilling and photoperiod in a series of experiments over a three-year period. The experimental materials were raised from seeds obtained throughout their extensive natural range including Canada, Alaska, and twenty-one states of the United States.

The experiments showed that photoperiod exercised the controlling influence over the continuation of shoot elongation and the onset of dormancy as shown by the terminal bud formation. There was no indication that the time of the initiation of growth was influenced by the photoperiod, except possibly in certain unchilled plants. The breaking of dormancy in certain unchilled plants seemed to be hastened by the exposure to long-day.

For most of the plants, winter chilling of 38°–40° F resulted in early and normal breaking of dormancy in spring. But under Florida conditions, plants of extreme northern origin broke dormancy without winter chilling; and with only a few exceptions all the unchilled plants eventually broke dormancy after various periods of delay. The leafing out of the unchilled plants, however, usually took place on the basal branches or near the base of the stem with the terminal bud remaining dormant for periods ranging from a few months to a year. Observations of the winter chilled plants showed that, with the exception of high altitude plants, the time of the breaking of dormancy and the time of the onset of dormancy were inversely correlated to the latitude of origin.

WARBURTON, DOROTHY. Factors in the Etiology of Spontaneous Abortions. *Department of Genetics, McGill University, Montreal, Canada.*

This study is based on data from the Family History files of the Department of Medical Genetics at the Montreal Children's Hospital. The purposes of the study are: (1) to make explicit the numerous sources of bias to which data collected on human spontaneous abortions are subject, and to assess their importance; (2) to

seek correlations which might elucidate some etiological factors in spontaneous abortions.

Some of the factors available for analysis are racial origin, maternal and paternal age, consanguinity, birth order, maternal menstrual history and previous reproductive history. The analysis of some of these is still in progress at the time of writing. So far it can be reported that abortion frequency is positively correlated with increasing maternal age and also with the number of live premature births. No relation has been found with racial origin, consanguinity, irregularity of the mother's menstrual cycle or the number of stillbirths in her reproductive history. Also, the frequency of abortion in families ascertained through a child with cleft lip or palate is significantly lower than in control families ascertained through a child without this defect. The relationship between abortion frequency and other malformations will also be investigated.

WARBURTON, FREDERICK E. Natural Selection as the Accumulation of Information from the Environment. *McGill University, Montreal, Canada.*

Shannon's mathematical theory of communication allows us to treat the gene complement of an organism as a "message." The information conveyed by the message to its recipient—in this case, to the developing organism—is (in binary digits):

$$H = \log_2 \frac{\text{probability of certain events after receipt of message}}{\text{probability of same events if message were not received}}.$$

In evolution, the information content of genomes has tended to increase. Mutation and drift can be shown to lead to loss of genetic information. The large information content of genomes must therefore have been accumulated by natural selection. The rate of accumulation, in binary digits/locus/generation could be calculated for a given population if mutation rates and variations in gene frequencies from generation to generation were known with sufficient accuracy; such calculations may be feasible within a decade.

By natural selection, the environment determines which genotypes will be transmitted to future generations; i.e., the original source of genetic information is the environment. Natural selection can therefore be considered as a mechanism by which populations accumulate information from their environments.

WATANABE, MICHIKO. *See* WATANABE, TSUTOMU.

WATANABE, TSUTOMU, and MICHIKO WATANABE. Transduction of Streptomycin Resistance in *Salmonella typhimurium*. *Department of Zoology, Columbia University, New York, N.Y., U.S.A.*

Although Zinder and Lederberg (1952) reported on the transduction of streptomycin resistance in *Salmonella typhimurium*, several workers have been unable to reproduce their transduction experiments on resistance to streptomycin as well as to other drugs. We have recently succeeded in transducing both streptomycin indifference (one-step complete resistance) and resistance in the narrow sense (multiple-

step resistance) using *Salmonella typhimurium* strain LT-2 and temperate phage P-22.

Transduction of indifference was found to require one to three generations for phenotypic expression. This lag may involve a segregation delay if the indifference locus is recessive in this strain. Phenomic lag is also assumed to play a role because transduction of indifference is phenotypically expressed gradually from partial to complete resistance, despite the fact that there is much evidence for the control of indifference by a single gene.

Transduction of intermediate resistance into sensitive bacteria does not give clear-cut positive results. The reason for this is unknown at present. The fact, however, that the second step of resistance may be transduced into one-step resistant bacteria suggests that the difficulty is merely technical. Furthermore, the substitution by transduction of the wild-type allele at the resistance locus was found to occur very easily with the following technique. Because resistant mutants are slow growers, it is easy to detect the fast-growing transductants on the poor background growth of the resistant recipient on agar plates without streptomycin. In transductions with one-step intermediate resistant mutants as recipients, the fast-growing transductants were found to be streptomycin sensitive. With this technique, it has become possible to cross resistance with sensitivity, resistance, and indifference. A number of independently isolated one-step and two-step resistant mutants have been analysed and many loci which control streptomycin resistance have been found. Linkage of streptomycin loci to other markers is also under study.

WATT, DEAN D., and H. WARNER KLOEPFER. Separation of Blood Proteins by Starch Gel Electrophoresis. *Southeast Louisiana Hospital, Tulane University, Mandeville, La., U.S.A.*

Smithies (Biochem. J. *61*: 629–641) developed a better method (zone electrophoresis in starch gels) for the resolution of alpha-beta globulins than had been obtained by other techniques. He found that certain electrophoretic patterns which were characteristic for the individual were controlled by genes.

The present study of electrophoretic patterns by the starch gel techniques was initiated in individuals of a large kindred in which a dominant gene for a pre-senile dementia was segregating. This study which was designed to learn more about the inheritance of serum proteins was facilitated by the development of a four-channel electrophoretic cell with separate electrical controls which will supply a constant current of 5.0 $\pm$ 0.1 milliamps. Samples of serum are run with the additions of hemoglobin (1.46 mg./ml.) and without the addition of hemoglobin.

Characteristic bands may be identified either by staining with amido-schwarz or by testing for the peroxidase activity of hemoglobin by the hydrogen peroxide-benzidin reaction. Serum proteins which bind hemoglobin seem to characterize the group of variations reported by Smithies. The techniques used in the present study and some of the results obtained will be discussed.

(This investigation was supported in part by a research grant, #B-962 (C2), from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.)

WEAVER, GERALD, M. *See* HOUGH, L. FREDERIC.

WEETH, H. J. *See* KIDWELL, J. F.

WEIJER, JAN. Evidence for a Complex Structure of the *td*-locus in *Neurospora crassa*. *Department of Plant Science, University of Alberta, Edmonton, Alta., Canada.*

Yanofsky's studies (1952, 1953) strongly suggested that the *td*-locus in *N. crassa* represents a complex structure consisting of several closely linked alleles. These alleles behave differently although they are alleles from a biochemical point of view since all are characterized by a lack of the enzyme tryptophan synthetase. The genetic analysis of these strains (Weijer, 1954) showed that several of the tryptophan independent recombinants recovered from intra-mutant crosses could not have originated from crossing-over within the *td*-locus, and hence genetic evidence for a complex structure of the *td*-locus could not be produced. In recent studies, several spontaneous *td*-mutants and one UV *td*-mutant (at low energy level) were obtained, all characterized by a tryptophan requirement which cannot be satisfied by indole. As a test of biochemical identity, heterocaryons between these strains could only be established in the presence of tryptophan. In six intra-mutant crosses using "fluffy" as a marker in one of the parental strains, tryptophan independent isolates were recovered from combinations involving *td*-UA2, *td*-UA5 and *td*-UA6. None of these strains produced tryptophan independent recombinants by selfing. The germination percentage of the ascospores in all the crosses was considerably higher (approximately 90%) than in previous experiments (50%). The results indicate that several closely linked genes are present within the *td*-locus, all of which must be present in the same linkage group for expression of the *td*-locus in tryptophan formation.

WEINSTEIN, ALEXANDER. Did Nägeli Fail to Understand Mendel's Work? *Biological Laboratories, Harvard University, Cambridge, Massachusetts, U.S.A.*

(See Appendix.)

WEIR, J. A., and H. G. WOLFE. Modifications of the Sex-Ratio of Mice through Genetic and Environmental Causes. *University of Kansas, Lawrence, Kan., U.S.A.*

Selection for blood-pH, with inbreeding, has given rise to a high blood-pH line of mice, pHH (7.476 ± .004) and a low line, pHL (7.426 ± .004). Concomitant shifts in sex ratio (pHH 55% ♂♂, pHL 45% ♂♂ cf. outbred T foundation stock 50% ♂♂) are permanent characteristics of the strains. Results from reciprocal crosses and artificial inseminations have established that the sex ratio is a function of sperm source. Sex ratios at third trimester and among stillborn mice do not differ significantly from the sex ratio of living mice at birth or weaning. Starting with descendants from the original outbred line, T, we are repeating the selection experiment, but with minimal inbreeding, and using arterial instead of venous blood. Results from three generations of selection are as follows: S_1 high line,

blood-pH 7.52 ± .007, sex ratio 48.3% ♂♂; low line, blood-pH 7.49 ± .007, sex ratio 57.6% ♂♂. S_2 high line, 7.56 ± .005, 47.3% ♂♂; low line 7.53 ± .005, 55.3% ♂♂. S_3 high line, 7.56 ± .005, 45.9% ♂♂; low line 7.51 ± .005, 50.0% ♂♂. Outbred mice contemporaneous with S_3 had blood-pH 7.53 ± .006, sex ratio 47.4% ♂♂. Significant changes in blood-pH of ♂♂ from inbred pHH and pHL strains, in the expected direction, have been produced by feeding acid and alkaline diets, but litters sired by treated males include significantly fewer male progeny, in each case, compared to sex ratios of litters from males on neutral diet. Although significant shifts in sex ratio result from changes in blood-pH brought about either by genetic or by non-genetic influences, the relationship is complex.

WELSH, J. N., and G. J. GREEN. Genes in Oats for Resistance to Stem Rust Races and the Genetics of Host Reaction. *Cereal Breeding Laboratory and Plant Pathology Laboratory, University of Manitoba, Winnipeg, Man., Canada.*

At the present time fifteen races of oat stem rust, *Puccinia graminis* Pers. f. sp. *avenae* Erikss. and Henn., have been identified on the North American continent. Four major genes (A, B, D, E) and probably a fifth (C) govern resistance to certain groups of these races. Gene A, possessed by the variety Richland, governs resistance to races 1, 2, 3, 5, 7, 7A, and 12. This is sometimes referred to as the Richland gene although a number of other varieties have the same resistance. Resistance to races 1, 2, 3, 5, 7, 12 and to races 4, 6, 8, 10, 11, 13, possessed by Hajira and its derivatives, is thought to be governed either by two closely linked genes, B and C, respectively, or by the B gene alone. In the United States this is referred to as the "Canadian factor" for resistance. Gene D, possessed by White Russian and its derivatives, governs resistance to races 1, 2, 5, 8, 8A, 10, 11, and gene E, possessed by Jostrain and its derivatives, confers resistance to races 1, 3, 4, 11 and partial resistance to races 5, 10, 12, 13 and 13A. Races 8A and 13A were isolated in Canada for the first time in 1957.

Results with several oat crosses confirm the existence of genes A, B, D and E, but throw some doubt on the existence of gene C. The crosses studied are as follows: Garry (ABC) × R.L. 231 (susc.); Garry (ABC) × Roxton (E); R.L. 1225 (BC) × Ajax (A); Canuck (BCE) × Victory (susc.); and Canuck (BCE) × Cherokee (D).

WELSHONS, W. J. Pseudoallelic Recessive Lethals at the *Notch* Locus of *Drosophila melanogaster*. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

The linear arrangement from left to right on the X-chromosome of three recessive visible mutants at the *Notch* locus has been established as follows: facet (*fa*), facet-notchoid (fa^{no}), and split (*spl*). Furthermore, experiments have indicated that the dominant *Notch* (*N*) mutants plus the recessive visibles constitute a pseudoallelic array similar to the star-asteroid and ultrabithorax-bithoraxoid series described by Lewis (1951). However, the Notch pseudoallelic array differs from the others in one important respect: there are at least three different sites capable of mutating to Notch, a recessive lethal condition, whereas in the other series only one member of the array is such a mutant. Since Notches are sex-linked recessive lethals,

this complex locus carries a pseudoallelic recessive lethal series and a recessive visible pseudoallelic array. The pseudoallelism of the Notch mutants was established on the basis of experiments outlined below.

N^{264-40}/spl and N^{Co}/spl heterozygotes, which also carried other appropriate markers, yielded spl^+ N^+ chromosomes; the majority of these were associated with recombination. If they arose by a cross-over between Notch and split, N^{264-40} had to be to the left of *spl* and N^{Co} to the right of *spl*. The following experiment supports this interpretation. Females which were $y\ w^a\ N^{264-40} + +\ /\ +\ w^a\ +\ N^{Co}\ rb$ yielded, by recombination between the two Notches, 13 chromosomes out of approximately 75,000 which were of the expected $+\ w^a\ +\ +\ +$ type (a small duplication carried by an autosome covers the lethality of the Notch combination). Additional experiments have identified another site, making a total of at least three, capable of mutating to Notch.

Thus, the Notches constitute the first pseudoallelic recessive lethals in *Drosophila*.

WETTSTEIN, DITER H. VON. Directed Mutability and Submicroscopic Gene Physiology in Chlorophyll Lethals. *Genetics Department, Forest Research Institute and Genetics Institute of the University, Stockholm, Sweden.*

Chlorophyll lethals in a self-fertilizing species such as barley are used as tools in the study of the mutation process in two ways: (1) as a test for the efficiency and quality of a mutagen; (2) for the genetic control of plastid development.

(1) The mutant spectra obtained after treatment with ionizing radiations and chemicals reveal considerable quantitative and qualitative differences in the action of mutagens. By applying chemicals we can differentiate between gene mutations and chromosomal rearrangements. Moreover, the mutation process can be concentrated at certain loci and chromosome breakage can be preferentially achieved in heterochromatic regions and other special structures of the chromosome.

(2) The nature of chlorophyll or plastid defects caused by gene mutations can be analysed by means of recently developed electronmicroscopic techniques. In the lethals investigated so far the synthesis of the lamellar structure in the chloroplasts is blocked at various stages, whereas the plastid growth as a whole is little or not at all affected. Examples will be given of certain genes controlling the formation of extended submicroscopic layers from smaller units. As a consequence of the blockage, structures of macromolecular size accumulate in certain mutants. Parallel chemical analysis enables us to isolate substances which might be contained in the observed submicroscopic structures. Plastids seem to be excellent material for studying the action of genes on the structural organization at a macromolecular level.

WHITEHOUSE, H. L. K. Patterns of Interference between Cross-overs in *Neurospora*. *Botany School, University of Cambridge, Cambridge, England.*

Analysis of asci segregating at four loosely linked loci (mating-type, *me* (35809), *al*-2, *nic*-1) in the mating-type chromosome of *Neurospora crassa* is in progress, with the object of obtaining information about the broad pattern of interference between cross-overs. Preliminary results are as follows. Across the centromere there appears to be negative position interference and an excess of two-strand relationships. This is similar to the findings of Lindegren and Lindegren (Genetics 27:

1–14) and Rifaat (Ph.D. thesis, Cambridge, 1956), and contrary to the results of Howe (Genetics *41*: 610–622) and Stadler (Genetics *41*: 623–630), who found no position or chromatid interference. Thus there appears to be heterogeneity in the patterns of interference across the centromere in different strains of *Neurospora*. In the proximal part of the right arm of the mating-type chromosome, the results indicate positive position interference and an excess of four-strand relationships. Similar though hardly significant results were found by Lindegren and Lindegren (*ibid.*). In the distal part of the right arm, there again appears to be positive position interference but no chromatid interference. This is similar to the results of Lindegren and Lindegren (Genetics *24*: 1–7) for another *Neurospora* chromosome.

Pritchard (Heredity *9*: 343–371) suggested that in *Aspergillus* crossing-over is confined to regions of "effective" pairing within which there is negative position interference, while the regions of effective pairing show positive interference with one another. A tentative explanation of the diversity in the patterns of interference in *Neurospora* would be to extend Pritchard's hypothesis and postulate that within regions of effective pairing only two-strand relationships occur between cross-overs, while between one such region and another, other strand-relationships are possible, perhaps as a consequence of delayed duplication of chromatids in the intervening regions. In some strains of *Neurospora*, one of the regions of effective pairing is presumed often to include the centromere.

WHITING, P. W., and SARAH B. CASPARI. Factor Homologies in Mutant *R* Genes of *Mormoniella. Department of Zoology, University of Pennsylvania, Philadelphia, Pa., U.S.A.*

Mutant genes in this series are unifactorial, bifactorial or trifactorial according to the number of factors affected by the mutation. The states or conditions of factors form subseries of homologous alternatives within the series of allelic genes. Homology is determined by the traits of the compound females. Thus for the 4 known eye-color factors *O*, *S*, *M* and *N*, the compound *o.s.*+.*n*/+.+.*m.*+ is wild type and +.*s.m.*+/+.+.*m.*+ is mutant type. Eye colors determined by the various combinations in the 4 subseries serve as markers for sterility or lethal factors. Thus wild-type female +.*da.la.*+/*oy.*+.+.*fsx* crossed to oyster male *oy.st.fsa.*+ produces sterile dahlia and fertile oyster daughters and fertile (female-sterile) oyster sons. Dahlia, *da*, is homologous with scarlet, *st*, and dominant. Female-sterile-a, *fsa*, is dominant over lethal-a, *la*, the two being alternative mutant conditions of factor *A*, but it is complementary with female-sterile-x, *fsx*, each condition being recessive to its normal alternative in the allelic gene. Sterility of compounds for different mutant genes bearing lethals with an *fsa* gene indicate that the lethals are homologous, affecting factor *A*. It has not hitherto been possible to test homology of two different lethals or of male-steriles directly because they could not be brought into the same cross. It now seems likely that this may be done on the diplo-triploid level. Diploid males have been crossed to females carrying a lethal. The daughters (triploid) with the lethal produce diploid sons, some of which are lethal-bearing. These are now being crossed to females with a different lethal. Half the triploid daughters should have both lethals and some of the diploid sons of these triploids should have both lethals unless the lethals are homologous. Failure to get males with both lethals will indicate their homology. (Work done under U. S. Atomic Energy Commission Contract AT(30-1)-1471.)

WHITING, P. W., GEORGE B. SAUL, 2ND, and DAVID T. RAY. *Mormoniella* and *Sarcophaga, University of Pennsylvania, Philadelphia, Pa.; Dartmouth College, Hanover, N.H.; Howard University, Washington, D.C., U.S.A.*

A demonstration will be given showing the mutant types of the chalcidoid wasp *Mormoniella vitripennis* (Walker) with living and preserved material. Charts showing methods of heredity, linkage maps and factorial formulae of genes will also be presented. The life history of the wasp including diapause larvae will be discussed.

Material on the blowfly *Sarcophaga bullata* Parker, including a discussion of its life history (living and preserved material), method of rearing and the parasitism of *Mormoniella*, will also be presented.

WHITTINGHILL, M., EVELYN E. HENDRICKS, GERVAS S. TAYLOR, JR., and LEWIS S. THORP. The Distribution of Rheumatoid Arthritis, Spondylitis and Multiple Rheumatoid Arthritis in a Single Large Kindred. *University of North Carolina, Chapel Hill, N.C., U.S.A.*

In a single kindred of some 3,000 persons it was found that cases of rheumatoid arthritis were distributed independently of spondylitis and multiple rheumatoid arthritis. Starting within the kindred from 28 rheumatoid arthritis cases additional cases of the same disease were found with higher incidence the closer the genetic relationship: one genetic step away, 3.7 $\pm$ 1.14%; at two genetic steps 2.4 $\pm$ 0.64%; at three genetic steps, 2.3 $\pm$ 0.60%; and only 9 of the 28 rheumatoids had no affected relative in this close a relationship. This pattern suggests a weak influence of heredity. The kindred was so extensive that the over-all incidence, 28/3,000, was as low as in a control area, where 3 rheumatoid cases and none of the other 2 were found among 337 persons. Among the 1,463 (overlapping) close relatives of rheumatoid arthritis cases there were only 2 cases of spondylitis. This was less than was to be expected for a random rather than a familial distribution; and, conversely, these same 2 pairs of relatives were fewer than chance expectation for rheumatoid arthritics among the total of 587 persons who were close relatives of the 10 spondylitics or of the 5 people with multiple rheumatoid arthritis. The spondylitics had higher than random frequencies of other spondylitics and of cases of multiple rheumatoid arthritis among their close relatives in a pattern suggesting a genetic foundation for this dual susceptibility. At one, two and three genetic steps removed the incidences were 6.3 $\pm$ 2.48, 2.9 $\pm$ 1.16, and 0.7 $\pm$ 0.5%, respectively. The 5 people with multiple rheumatoid arthritis had as close relatives people with spondylitis rather than with other forms of juvenile or adult peripheral arthritis, even though all 43 cases were remotely related. Although the genetic basis acts weakly, it seems to differentiate a tendency to multiple rheumatoid arthritis and to spondylitis in children and youths as one genetic entity, which is separate from an inherited tendency to rheumatoid arthritis at later years.

(This work was aided in part by research grants A-26 and A-53 from the National Institute of Arthritis and Metabolic Diseases, U. S. Department of Health, Education and Welfare, and by C-136 from the Carnegie Research Fund of the University of North Carolina.)

WILCZYNSKI, JAN Z. Orthogenesis as a Genetical Problem. *Lebanese State University, Beirut, Lebanon.*

Refuting the autogenic and selectional explanations of the phenomena of orthogenesis, the author seeks to explain them by microevolutionary processes, and so provide a possible, though restricted, solution in terms of the mechanisms of hereditary transmission. Since analysis and confirmation of his theory from the point of view of population genetics is not yet possible, he limits himself to the genotypical analysis of expected sequences of produced zygotes as individualized entities.

The author's argument is based on the general equation of Mendel's Law in their genotypical representation, developed by him in 1938–51 as an expansion of a binomial formula: $P_{\blacktriangle}\, 2^m\, 2^{n-m}$, where $P_{\triangle}$ is Pascal's arithmetic triangle, m is a series of natural numbers, and n is an allelic power of any crossing. In this expression the zygotes are presented by particular and different terms with regard to both their genotypical components and their respective frequencies. The phenomena of orthogenesis would then correspond to the terms giving the fullest and strongest integration of the features (genes) concerned, together with their greatest frequencies. Since these will tend to be perpetuated through the generations, such organisms ought to acquire ever greater chances of survival by securing stronger expression and more prolific reproduction with each succeeding generation, which may result in apparent orthoselection.

His point of view is discussed with examples taken from studies of polypoidy, of polymerism, of gene interactions and heterosis, of gradual chemical complication in the physiology of digestion and growth, and of the phenomena of mimicry and of biogeographic variability. He considers the possibility of applying to all of these examples the genotypic interpretation of orthogenesis.

WILKINSON, CHAS. F., Jr. *See* HIRSCHHORN, KURT.

WILLIAMS, L. F. Alteration of Dominance and Apparent Change in Direction of Gene Action by a Mutation at Another Locus Affecting the Pigmentation of the Seedcoat of the Soybean. *U.S. Department of Agriculture,* and *Department of Field Crops, University of Missouri, Columbia, Mo., U.S.A.*

Major pigmentation patterns of the seedcoat of the soybean are controlled by a series of alleles at the *I* locus. On a *TR* background (tawny pubescence, black pigment), the effects of these alleles, arranged in decreasing order of dominance, are as follows: *I*, pale slate hilum, coat yellow or green; i^i, black hilum, seedcoat yellow or green; i^k, hilum and saddle black, remainder brown; *i* self black. Ordinarily, dominance is complete.

In two mutants from i^i types, the hilum and saddle are black with the remainder yellow or green. This is a result of a recessive mutation at the *K* locus, the effect of *k* being to increase pigmentation. However, the other three *I* alleles, which have very distinct effects on a *K* background, have identical phenotypes in combination with *k*. The homozygous types are all self black and the i^i heterozygotes have an extended saddle closer to self black.

The *I* allele is affected most. In combination with *K*, *I* seems to have a strong inhibitory effect, but with *k* it seems to have an enhancing effect. This change in dominance and in type of allele action is difficult to explain. A suggested explanation is that *I* rather than inhibiting pigment, merely has a very weak enhancing effect and *k* is thus able to affect pigmentation.

WILLIAMS, WATKIN. The Manifold Effects of Major Genes in Relation to Economic Characters in Plants. *John Innes Horticultural Institution, Hertford, England.*

Studies of the genetic control of flower number in genotypes homozygous for the suppressed lateral gene *ls* will be described. The analysis of F_2 families segregating for recessive marker genes showed that when the marker was present in the homozygous recessive condition the effect of *ls* in reducing flower number was intensified. The most pronounced effect was produced by interaction between *ls* and *dm* (linkage group IX) and *ls* and *l* (linkage group VI). The only interactions which effected an increase in flower number above *ls ls* + + were *ls ls a a* (linkage V) and *ls ls y y* (linkage group III). None of the ten markers proved completely neutral in respect of flower number when combined with *ls*. When scored singly the major gene markers differed insignificantly from the dominant wild type except in the genotypes *wt wt* and *b b* where a significant increase over the wild type was demonstrated.

Data in respect of four loci in *Prunus persica* and *Prunus avium* will also be briefly presented. The neutrality of major genes in respect of character expression will be discussed, and their importance in plant breeding assessed.

WILLIAMS, WATKIN, and G. J. DOWRICK. The Induction of Mutations in Plants Using Radio-active Phosphorus (P^{32}). *John Innes Horticultural Institution, Hertford, England.*

The mutagenic effect of P^{32} on three plants—the apple, tomato and foxglove (*Digitalis purpurea*)—has been studied at the John Innes Horticultural Institution. Different methods of administration were compared and it was found that injection of the P^{32} was most satisfactory. The distribution of radio-activity in various parts of the plants was measured and the biological half-life of the isotope was found to be substantially less than its physical half-life owing to the exudation from the roots into the soil.

In the apple the efficiency of the dose is directly related to the number of active buds. At higher bud numbers a higher proportion of the injected dose was recovered in the growing points. Thus the same specific activity can be obtained in a tree with 96 buds, as compared with a tree with 12 buds simply by doubling the dose administered.

The upper level of tolerance of the vegetative buds of the apple was found to be 50μC/gm. dry weight. This corresponds to a dose of 44,500 rep. assuming total absorption of the particles. If the absorption is 50% and the biological half-life is half the physical life, the calculated dose at the upper tolerance limits will be reduced to 11,000 rep.

Mutant phenotypes identical with those existing in wild populations have been recovered. However, mutant plants may be characterized by differences in growth rates as compared with both the wild type and the naturally occurring mutants. Evi-

dence is presented which indicates that the variability of complex quantitative characters is increased in irradiated material and that selection for maximum character expression is therefore made more effective.

WILLIAMSON, D. L. Observations on Carbon Dioxide Sensitivity in the *Drosophila affinis* Subgroup. *University of Nebraska, Lincoln, Neb., U.S.A.*

Carbon dioxide sensitivity (death in CO_2 at 13°C) has been found in wild-caught *Drosophila affinis* and *athabasca*, and also in laboratory strains of *affinis*, *athabasca*, and *tolteca*.

D. affinis collected in Nebraska during the summer of 1957 exhibited 25% sensitivity, and *athabasca* collected in Alaska during July and August, 1957, showed 8.5% sensitivity.

A laboratory strain of *affinis* from Nebraska was found to be nearly 80% sensitive, and a strain of *athabasca* from Alaska was 99% sensitive. In addition, laboratory strains of *tolteca* from Nicaragua and Mexico were found to exhibit 5% and 8% sensitivity respectively.

Reciprocal matings involving the sensitive *athabasca* strain and a resistant strain of this species have indicated that the sensitivity is maternally inherited.

WILLWEBER, EN. *See* YAMASHITA, KOSUKE.

WILSON, G. B., A. H. SPARROW, and VIRGINIA POND. Radiation-Induced Sub-Chromatid Breakage and Rejoining during Meiosis of *Trillium erectum* L. *Brookhaven National Laboratory, Upton, Long Island, N.Y.*, and *Michigan State University, U.S.A.*

Anthers of *Trillium erectum* were X-irradiated with 25 r at various stages of meiotic prophase and at the first metaphase. Analysis of the aberrations at the first and second anaphases revealed that pre-diplotene treatment did not and that post-pachytene irradiation did produce configurations (2-side-arm bridges) indicative of half-chromatid exchanges. The occurrence of these bridges and knowledge of the structure and spatial relationship of chromatid strands in *Trillium* have led to certain conclusions regarding (1) the target and (2) the number of sub-chromatid strands broken by a single event. (1) The most likely targets for primary effects are the four associated half-chromatids of a half-bivalent. The irradiation results suggest that the half-bivalent becomes effectively as well as structurally quadripartite at stages following pachytene. (2) Consideration of the configuration which would result from breakage and rejoining of two-, three- or all four-strands of the half-bivalent indicates that only two of the four half-chromatids are broken by a single event, i.e., a single ionization track. Exchanges between these two half-chromatids will produce two recognizable types of 2-side-arm bridges: one with a true dicentric half-chromatid and one in which the bridge results from an interlocking of coils. Whether a 2-side-arm bridge will appear at first or second meiotic anaphase is determined by the position and number of chiasmata between the point of breakage and the kinetochore. So far no 2-side-arm bridges have been detected

at microspore anaphase from meiotic prophase irradiation. The types of configurations which might be expected at microspore metaphase as a result of broken 2-side-arm bridges have been observed but at a very low frequency. (Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.)

WOLF, B. ERICH. Control of the Crossing-Over Frequency in the X-Chromosome of *Phryne cincta* by Allocycly (Diptera: Nematocera). *Institut für Genetik der Freien Universität Berlin, Berlin, Germany.*

A remarkable correlation between a genetic feature and the allocyclic behaviour of the X-chromosome in *Phryne cincta* has been discovered.

As to the allocyclic behaviour of the X-chromosome, the giant chromosomes of *Phryne cincta* ($n = 3 + X(Y)$) from larvae raised at different temperatures have been studied (Wolf, Chromosoma *8*: 396). The X-chromosome is characterized by a considerable range of temperature-dependent variability in shape and structure, caused by its allocycly. Under ordinary room-temperature conditions it is a flat granular body, broader but relatively much shorter than the autosomes. At low temperature it grows to normal length, assuming the aspect of a normal giant chromosome. The temperature-conditioned variability of the X is a new expression of its allocycly, previously observed only in mitosis and meiosis.

As to the genetic behaviour of the X-chromosome, a considerable range of temperature-dependent variability in the frequency of a localized crossing-over, correlated with the allocyclic behaviour of the X in somatic cells, could be demonstrated. For this purpose female animals—heterozygous for several structural rearrangements in their X-chromosomes and raised either at high (20–26°C) or at low (2–8°C) temperatures— were mated with males of a definite race. The female progeny of 19 single pairs were tested by analysis of their giant chromosomes. Besides the expected types of X-chromosomes there were found two complementary types of recombination caused by a strictly localized crossing-over between the starting types. In all 1,432 X-chromosomes have been tested. Only a small rate of crossing-over or none at all was found (mean 3.2%) in the "warm-raised" hybrids in contrast to their "cold-raised" sisters who showed a high frequency (mean 21.15%).

The conditions causing the structural modification of the X-chromosome in somatic cells are evidently the same as those which cause the variation in the frequency of the localized crossing-over.

WOLFE, H. G. *See* WEIR, J. A.

WOLFF, GEORGE L., and BERTON F. HILL Impact of a National Cancer Chemotherapy Screening Program on Mammalian Genetics in the U.S.A. *National Cancer Institute, N.I.H., U.S. Dept. of Health, Education and Welfare, Bethesda, Maryland,* and *Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, D.C., U.S.A.*

One million (C57BL/6 × DBA/2)F_1 hybrid mice per year are needed by the screening program for anti-cancer substances of the Cancer Chemotherapy National

Service Center (National Cancer Institute, U. S. Public Health Service). This unprecedented demand for mice of a specific genotype provided the stimulus for the development of several national programs in the areas of mammalian genetics and laboratory animal production.

A set of "Minimum Standards for the Commercial Production of Randombred and Inbred Laboratory Mice" (J. Nat. Cancer Inst. *19*(6): 1137–1143) was developed by the Institute of Laboratory Animal Resources (National Academy of Sciences) under a contract with the Cancer Chemotherapy National Service Center. These "Minimum Standards" are serving as the basis for the accreditation of commercial mouse producers in the U.S.A.

Several commercial breeders were chosen on the basis of technical competence, compliance with the "Minimum Standards," and geographic location to receive C57BL/6 females and DBA/2 males from the Jackson Memorial Laboratory on a replacement basis for the production of a large part of the one million (C57BL/6 × DBA/2)F_1 hybrids.

With the assistance of grants-in-aid and research contracts from the National Cancer Institute of the U. S. Public Health Service, centers for training and research in mammalian genetics have been established at the Universities of Kansas, Miami, and Michigan. The inbred mouse colonies at these centers will also provide insurance stocks of inbred mouse strains available in case of disaster at one or more of the mammalian genetics centers, including the Jackson Memorial Laboratory. In addition any large-scale future needs for inbred or F_1 hybrid mice of specific genotypes can be met more expeditiously by the establishment of additional "satellite breeders" receiving breeding stock on a replacement basis for one or more of the mammalian genetics centers.

WOLFF, SHELDON. Protein Synthesis and Chromosome Break Rejoining. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.*

If the dose of radiation administered to the soaked seed of *Vicia faba* is fractionated, the frequency of chromosome exchanges induced will vary according to the fractionation time. If the breaks from the first dose have not rejoined before the second dose is administered, the aberration yield will be the same as it would have been if the dose had not been fractionated (proportional to the square of the total dose). If the breaks from the first dose have rejoined completely before the second dose, then the yield will be the sum of the yields of the partial doses. Previous experiments have shown that the rejoining of the breaks is not a passive process, that cellular metabolism and the production of ATP is necessary for breaks to rejoin, and that the breaks are of covalent bonds.

In order to elucidate the mechanism of rejoining, a dose fractionation study was performed in which the breaks from the first dose would rejoin in one-half hour. Immediately after this first dose, the seeds were treated with 300 /μg./ml. of chloramphenicol to inhibit protein synthesis. The breaks then did not rejoin but remained open for at least seventy-five minutes as determined by the aberration yield when a second dose was administered seventy-five minutes after the first.

The results of these experiments constitute presumptive evidence that protein synthesis is necessary for the rejoining of radiation-induced chromosome breaks. As yet it has not been determined if the bonds synthesized during rejoining are peptide bonds or if protein synthesis acts more remotely in the repair of damage to a postulated "rejoining system."

Wolsky, Alexander. The Metabolic Basis of Some Gene-Controlled Characters in *Drosophila* and *Mormoniella*. *Biological Laboratory, Fordham University, New York, N.Y., U.S.A.*

Three gene-controlled character pairs of *Drosophila melanogaster* and one of the parasitic chalcid wasp *Mormoniella vitripennis* will be considered. "Ebony" of *D. melanogaster* differs from its wild-type allele in darker imaginal integument and paler (and softer) puparium. On the basis of earlier investigations of the author and others (Csik, Dennell, Fabian, Ohnishi) and unpublished new results, an attempt will be made to correlate the differences in enzyme activity (dehydrogenases, tyrosinase). A sex-linked genetic mechanism (discovered in an inbred population from Java), which is not yet fully clarified, affects the number and arrangement of interocellar bristles (Mulrennan). Its expressivity can be modified by reducing the food supply of the larva and this together with other evidence indicates that the gene or genes involved affect metabolic processes. Eye color in *D. melanogaster* is known to depend on genes which are closely associated with enzyme reactions, leading to the formation of red and brown pigments. Experiments show that these reactions are not specifically inhibited by the characteristic poison of the cytochrome oxidase system, carbon monoxide. On the other hand, in *Mormoniella* the formation of the brown eye pigment is specifically inhibited by carbon monoxide (Rohner) and treatment with a carbon monoxide–oxygen mixture during metamorphosis results in specimens with red eyes, which are phenocopies of the "scarlet" mutation described by Whiting. Thus the metabolic basis of eye pigment formation is different in the two species.

Woods, Philip S., and J. H. Taylor. Nucleic Acid Synthesis in Dividing Cells. *Brookhaven National Laboratory, Upton, N.Y., U.S.A.*

The metabolism of RNA and DNA in dividing cells has been studied by autoradiograph tracer techniques. Seedlings of *Vicia faba* were grown on nutrient solutions containing tritium-labelled cytidine a precursor of RNA and DNA; and at intervals up to 8 hours roots were fixed, and sections were covered with autoradiograph stripping film.

Autoradiographs showed that incorporation of the labelled precursor took place first in the nucleolus and later in the cytoplasm. Radioactivity in the chromatin of nuclei was variable; some nuclei showed strong autoradiographs, others showed weak or no autoradiographs.

From a separate chromatographic study using non-labelled roots, it was found that no RNA or DNA was lost prior to attachment of the photographic emulsion to the sections. RNA could be quantitatively removed from sections without loss of DNA. Upon using these procedures on H^3-labelled roots, it was found that radioactivity in the chromatin of nuclei was due largely to incorporation into DNA. Radioactivity in other parts of the cell was due to incorporation into RNA.

To determine whether the H^3-cytidine was incorporated in both the nucleolus and cytoplasm either with or without turnover, or was incorporated largely in the nucleolus and later transferred as a large molecule to the cytoplasm, other seedlings were treated with H^3-cytidine long enough that only nucleoli were labelled and then were removed and grown on a non-labelled solution containing an excess of cytidine and uradine. Autoradiographs showed a decline in radioactivity in nucleoli and a rise in the cytoplasm with increased post-treatment time.

The nucleolus either is the only site of RNA synthesis in the cell, or is concerned with accumulating RNA that has been synthesized elsewhere, but the synthesis time is very short. RNA as a large molecule is then transferred to the cytoplasm. DNA and RNA synthesis are independent processes.

WOODWARD, D. O. Heterocaryon Complementation between Adenylosuccinaseless Mutants of *Neurospora crassa*. *Botany Department, Yale University, New Haven, Conn., U.S.A.*

Thirty-five adenine-requiring adenylosuccinaseless mutants at the *ad-4* locus of *Neurospora crassa* have been tested for heterocaryon complementation in all possible pair combinations. Fourteen of the thirty-five mutants gave at least one positive response. In all cases tested in which complementation occurs, partial restoration of adenylosuccinase activity has resulted. Enzyme activities of the crude mycelial extracts (tested by following the splitting reaction of adenosine monophosphate succinate to adenylic acid in a recording spectrophotometer) range from 3% to 30% of that found in the parental wild type strain, 74A. Based on the complementation pattern, it has been possible to arrange these *ad-4* alleles in a linear sequence. This can be done because of apparent overlap between non-functional regions characteristic of the individual alleles. Meiotic recombination occurs between some of the alleles, giving rise to wild-type progeny. Based on the complementation pattern, these appear to be the more widely separated alleles of the linear sequence. Furthermore, the order of the alleles deduced from such crosses utilizing linked markers on either side of the locus does not conflict with the order based on the heterocaryon tests. To date, infertility in many of the crosses has made a complete recombinational analysis impossible. Certain heterocaryons are temperature sensitive in that they exhibit essentially wild-type growth at 25°C and little or no growth at 35°C. There is some indication of a specific region or regions within the locus affecting temperature sensitivity. (Supported in part under a contract AT(30–1)–872 with the U.S. Atomic Energy Commission.)

WOODWARD, V. W. *See* MUNKRES, K. D.

WOODWARD, VAL W., YOSHITAKA SUYAMA, and K. D. MUNKRES. New Loci Affecting Pyrimidine Metabolism in *Neurospora*. *Kansas State College, Manhattan, Kan., U.S.A.*

From over one hundred pyrimidine-requiring mutants of *Neurospora crassa*, recently isolated in our laboratory, at least two are genetically and physiologically distinct from any reported to date. In an earlier publication, a fifth locus involved in pyrimidine biosynthesis was implied, but the evidence was equivocal. It is now evident that the earlier implication was based on the phenotype of a double mutant, KS-12. Mutant KS-12 is physiologically similar to pyr-3 (37301), but is genetically dissimilar. On crossing, it gives rise to two distinctly different mutants, one of which is designated as pyr-5, and the other, laggard (lg), a pseudoallele to pyr-1 (263). In a cross between KS-12 and KS-43 (a pseudoallele of pyr-3), from one ascus,

ascospore pair 3–4 was lg. Mutant lg is physiologically similar and pseudoallelic to pyr-1 (showing 0.53% wild-types). Pyr-1 and lg grow slightly in minimal medium and are stimulated by uridine, uracil, dihydrouracil and orotic acid; pyr-1 responds to β-ureidopropionic acid, but lg does not. From the same ascus (KS-12 $\times$ KS-43), ascospore pair 7–8, when crossed to wild-type, gave rise to pyr-5. Pyr-5 is physiologically similar to lg except that it does not grow at all in minimal medium; it responds to the same, but not to different, metabolites. Mutant pyr-5 appears to be located distal to pyr-3. Its physiological requirements lead us to conclude that its mutant effects involve the reaction β-UPA $\leftrightarrow$ DHU in the uracil reductive pathway. Mutants involving metabolic interruptions of several steps in the reductive pathway of uracil metabolism as well as the pathway of orotic acid biosynthesis have been studied. The over-all metabolic-genetic scheme will be presented. (This research was supported by a grant from the National Institutes of Health, RG-4561 (C2) Contribution No. 616, Dept. Agron., Kansas Ag. Expt. Sta.)

Wright, James E., and Keen Buss. Barriers to Artificial Hybridization of Certain Species of the Salmonidae. *Pennsylvania State University* and *Pennsylvania Fish Commission, University Park, Pa., U.S.A.*

Although certain trout species occupy the same bodies of water and although their spawning periods overlap, natural species hybrids are extremely rare. Recent attempts to artificially hybridize these species for special-purpose hatchery production fish and in order to transfer traits from one species to another by backcrossing revealed a number of isolating mechanisms which limit or prevent hybridization.

The trout species involved, with their somatic chromosome numbers, were: lake trout, *Salvelinus namaycush*, 84; brook trout, *Salvelinus fontinalis*, 84; brown trout, *Salmo trutta*, 80; and rainbow trout, *Salmo gairdneri*, 60. Reciprocal matings between these species were made using several representative males and females from each.

Length of the incubation period of the eggs of the parent species was directly related to hatchability. The incubation period of rainbow trout eggs is approximately 10 days shorter than that of eggs of the other 3 species. When eggs of the latter were fertilized by sperm from rainbows, embryos formed and reached the eyed stage but never hatched. When eggs of the rainbow were fertilized with sperm from any of the other 3 species, some hatchability occurred but the newly hatched alevins appeared very immature and almost all soon died.

Egg size plays an important part in the condition and the survival of sac fry. For example, matings of brook trout females with lake trout males result in progeny extremely crippled in the caudal region. In other matings crippling occurred in the notochord in the dorsal fin region.

Inability to absorb the yolk sac resulted in severe losses in alevins of brown female $\times$ brook male, and of rainbow female $\times$ brook male crosses. Non-pathogenic white-sac and blue-sac conditions developed in the sac fry.

Hybrids from lake trout female $\times$ brook trout males are relatively highly fertile. In the few hybrids that reach maturity from other crosses limited gonad development leads to very high degrees of sterility.

WRIGHT, THEODORE R. F. The Phenogenetics of the Embryonic Lethal, *1*(1)48j, in *Drosophila melanogaster*. *Yale University, New Haven, Conn., U.S.A.*

The recessive embryonic lethal, *1*(1)48j, is located at 27.7 ± 0.2 on the X chromosome. Lethal hemizygotes never hatch and invariably show two characteristic abnormalities. The most obvious is a mid-dorsal herniation through the hypoderm which includes variable amounts of brain and/or mid-gut. The hypoderm bordering the extruded material becomes heavily chitinized. All of the musculature of the embryo, somatic, visceral, and pharyngeal, is abnormal. Instead of the typical long, slender muscles of the normal embryo one finds large spheroid masses with tremendous increases in volume. This muscular enlargement is so great that the hemocoel is almost completely obscured. These muscles do contract, but do not do any effective work. Although the muscles are still attached to the hypoderm by fine processes there is no muscle tone because of the increased size. No peristalsis has been observed. All other tissues and organs are normal except the mid-gut. Abnormalitites of the morphology of the mid-gut are variable and are dependent on the amount of mid-gut extruded in the herniation.

Lethal embryos are indistinguishable from normals up to 14 hours of development (25°C). Myogenesis proceeds normally and dorsal closure is completed. Between 14 and 14.5 hours the lethal embryos split open along the dorsal mid-line and brain and/or mid-gut is extruded. At the same time the first changes in muscle size and shape are seen. The muscles start to take on the spherical shape by a gradual enlargement of the middle portion of muscle while the ends toward the apodemes begin to thin out. The muscles rapidly become spheroid and continue to increase in size until the death of the embryo.

Histochemical stains for DNA, RNA, carbohydrates, lipids, and proteins do not reveal any qualitative differences between normal and abnormal muscles. Implantation of abnormal embryos into third instar larvae shows that growth and differentiation can continue and that the lethal is physiologically capable of molting. (This investigation was supported by a Predoctoral Fellowship, CF–6730, from the National Cancer Institute, Public Health Service.)

WYLIE, ANN P., and A. J. S. DAVIES. Giant Chromosomes and Amitosis in the Endosperm of *Lathyrus*. *Department of Botany, University of Manchester, England.*

The development of embryo (including the coenocytic middle-piece and suspensor cells) and endosperm in *Lathyrus* can be followed from two to three days after fertilization onwards, by dissection of intact embryo sacs from ovules stained with the Feulgen reagent. The curved cytoplasmic sac containing the endosperm nuclei can be opened and spread into a single sheet, yielding preparations suitable for detailed cytological study. In the normal course of development, the nuclei in the chalazal region of the endosperm undergo endomitotic growth and attain very high levels of ploidy. Such polyploid nuclei can subsequently divide by normal mitosis and also by an amitotic process, in which their chromosomes are partitioned approximately equally between the two daughter nuclei. These daughter nuclei also can subsequently undergo a normal mitotic cycle. Their origin is revealed by their

varying chromosome numbers and by the presence of small acentric rod and ring chromosome fragments, derived from chromosome breakage during the amitotic process. Micronuclei are generally common in regions of endosperm where amitosis has occurred at an earlier stage in development.

Giant polytene chromosomes also arise in the chalazal nuclei, from about 14 days after fertilization onwards. Nuclei with giant chromosomes may undergo amitosis but not a normal mitosis. The regularity with which giant chromosomes occur varies from species to species, depending probably on their heterochromatin content.

Preliminary examination of some other members of the Papilionaceae indicates that the situation found in *Lathyrus* is widespread in the family.

In some interspecific crosses in *Lathyrus* where the embryos abort before reaching maturity, various aberrations of endosperm development occur. These aberrations frequently involve failure of differentiation of the chalazal region of the endosperm. In other cases, endosperm development appears quite normal, and the primary cause of failure resides in the embryo itself.

YAMADA, YOSHIRO. Heredity in Congenital Deafmutism and Hereditary Inner Ear Deafness. *Tokyo Metropolitan Police Hospital, Tokyo, Japan.*

It is known that the three outstanding hereditary diseases in the otological field are congenital deafmutism, hereditary inner ear deafness and otosclerosis. However, in Japan, otosclerosis is seldom seen. This subject has been of particular interest to me for a long time and resulted in a study of forty families.

The first few cases of those I am presenting are of congenital deafmutism caused by consanguinity.

In Family I congenital deafmutism was found in 6 of the 7 siblings. Such a high frequency is rarely seen. It is interesting to note that of the 6 siblings 3 had other neuro-psychiatric diseases, such as epilepsy, depression, and insanity. Family II presented 3 cases of congenital deafmutism. The fact that both husband and wife had perfectly normal children by their previous marriages is worth mentioning.

Family III with 4 siblings included triplets all of whom had congenital deafmutism. From the point of view of heredity it is not necessary to state that all three families had recessive transmissions.

Families IV, V, and VI represent hereditary inner ear deafness. Family IV presented a typical case not only manifesting itself in four generations in succession but appearing in many instances in every generation. The degree of deafness in these cases was from slight to complete. Family V also consanguineous revealed that out of 7 siblings 5 had inner ear deafness varying in degrees. In Family VI the parents were aunt and nephew, though such a marriage is not legal. Of the 7 children, 2 died at an early age, 1 was a deafmute, 2 had labyrinthine deafness, retinitis pigmentosa and coloboma iridis, and 2 were normally healthy.

From this it may be possible to conclude that hereditary labyrinthine deafness has two transmission types, a dominant and a recessive often complicated with other ophthalmological or neurological defects. In the last two families mentioned the sclera were blue which seemed to be of a dominant transmission since they were seen in 3 to 4 generations simultaneously, accompanied by inner ear deafness and osteopsathyrosis.

YAMAMOTO, TOKI-O. Progenies of Induced Sex-Reversal Females Mated with Sex-Reversal Males in the Medaka, *Oryzias latipes*. *Biological Institute, Faculty of Science, Nagoya University, Chikusa, Japan.*

Success in inducing functional sex-reversal in both genetic males(XY) and females(XX) of the medaka (or killifish) under the influence of heterotypic hormones rendered it possible to mate sex-reversals with sex-reversals. The sex-linked *r*(white) and *R*(orange-red) genes were used as markers for the sex-chromosomes, using as material offspring of white females(X^rX^r) mated with heterozogous orange-red males(X^rY^R) which display the father-to-son inheritance. Induction of sex-reversal in genetic males was made under the influence of estrone and in genetic females by administration of methyl testosterone. Besides matings between sex-reversals(4), the following three kinds of matings served as controls: (1) crosses of normal X^rX^r females with sex-reversal males(X^rX^r); (2) matings of sex-reversal females(X^rY^R) with normal X^rY^r males and (3) matings of normal X^rX^r females by both X^rY^r and X^rY^R males. Eight crosses of (1) yielded all female progenies. Five matings of (2) resulted in offspring in agreement with the expectancy of 1 *r* ♀ : 1*r* ♂ : 2*R* ♂ ♂, indicating that Y^RY^r males are by no means rare. This fact is in strong contrast to the rarity of Y^RY^R males. Three matings of (3) produced offspring of the two sexes in equal numbers. Three matings(4) of sex-reversal females(X^rY^R) by sex-reversal males(X^rX^r) resulted in 309 *r* ♀ ♀ and 302 *R* ♂ ♂ with 5 exceptional *r* ♂ ♂ and 9 exceptional *R* ♀ ♀. Two exceptional *r* ♂ ♂ and three exceptional *R* ♀ ♀, successfully tested, proved to be crossovers. Crossing-over between the X^r and Y^R does take place in induced sex-reversal females with the heterogametic constitution X^rY^R. The crossover value in X^rY^R females is found to be significantly higher than in normal X^rY^R males.

YAMASAKI, YOSHITO, and MASAHITO KIKUCHI. Embryo Transplanting as a Method of Genetico-physiological Study in Cereals. *National Institute of Agricultural Sciences, Hiratsuka, Kanagawa, Japan.*

Since 1932 an embryo transplanting experiment has been conducted with mature seeds of cereals (wheat, barley, rye, maize and rice). In 1951 a new technique was developed by which an immature embryo in the course of its development is transplanted into an immature endosperm in order to carry out a genetico-physiological study of the influence of the endosperm on the expression of characteristics of the transplanted embryo.

An endosperm of non-dormant or after-ripened seeds has the ability to promote the germination of an embryo of dormant or intermediate seeds transplanted into the former, but an endosperm of dormant seeds does not always inhibit the germination of the embryo of non-dormant, after-ripened, or intermediate seeds. From these facts the following hypothesis was suggested. The embryo and the endosperm of dormant seeds contain a large amount of germination-inhibiting substances, and little or no germination-promoting substances, while the embryo and the endosperm of non-dormant seeds contain a large amount of the promoting substances, and little or none of the inhibiting substances. Intermediate seeds contain the two kinds of substances in various ratios according to the degree of dormancy. The inhibiting substances may be converted into the promoting substances by treatment with low temperature and oxygen, and by after-ripening.

An endosperm of a spring variety transplanted to an embryo of winter variety does not exert any influence on the flowering behaviour. The same is true of the reverse transplantation. An excised embryo of winter variety did not produce an ear when treated by low temperature before its transplanting to an endosperm, but when treated after its transplanting it produced an ear even when the endosperm of spring or winter varieties, or the endosperm of different species (Einkorn and Emmer wheat) and genera (barley, rice and maize) were used. The supply of some substances (perhaps carbohydrates) from an endosperm during low temperature treatment seems to be necessary to flower formation of an embryo of a winter variety.

Although it is not yet clear what changes do occur at the growing point of the embryo thus treated, the authors suppose that there may be changes in the enzyme systems, induced by the low temperature treatment, due to the presence of carbohydrate, oxygen etc.

YAMASHITA, K. *See* KIHARA, H.

YAMASHITA, KOSUKE, and EN WILLWEBER. Studies on X-Ray Induced Mutations in Einkorn Wheats. *Biological Laboratory, Kyoto University, Kyoto, Japan.*

Dormant seeds of *Triticum aegilopoides* and *T. monococcum* were treated by X-ray, and a number of morphological and physiological mutations were obtained. Those materials have been submitted to cytological and genetic investigations and the data will be presented.

YARNELL, S. H. Penetrance and Expressivity of Six Characters of Cabbage. *U.S. Southeastern Vegetable Breeding Laboratory, Charleston, S.C., U.S.A.*

Terminal Notch is limited to one or a few leaves per plant. Maximum percentage of Notch plants in inbred lines was 2.7 (1955) to 20.5 (1950). Four commercial varieties produced 1.8 to 3.7% Notch (1956) while selected Notch from two varieties produced 3.7 and 4.5%. Selection increases the proportion of Notch, but penetrance and expressivity are low. Populations segregating Notch frequently produce Heterophyllus, a very irregularly shaped leaf. Maximum percentage of Heterophyllus ranged from 7.5 (1957) to 34.9 (1953). Heterophyllus is restricted in both penetrance and expressivity but less so than Notch.

The other four characters affect the entire plant. Pseudowhiptail, certain features of which resemble whiptail of cauliflower, develops late, when the inner leaves become misshapen with very coarse serrations, and the plant becomes lopsided. Maximum percentage varied from 17.5 (1954) to 88.1 (1951). Pseudowhiptail families may also produce Poorkin, a small semi-lethal. Poorkin leaves are thick, often long, and sometimes curled. Maximum percentage of Poorkin ranged from 19.3 (1953) to 73.4 (1957).

Balloon Rosette and Flat Rosette are descriptive of these types. Maximum percentage of the former in inbreds ranged from 1.3 (1954) to 96.4 (1952) and of the latter from 5.0 (1952) to 94.8 (1957).

Environmental conditions may affect the proportions of these six types each

season, but expressivity remains unaffected. Environmental effect is gauged by the seasonal range of maximum penetrance of each character. Replicates grown in different years remain essentially unchanged when proportions are less than 10%. For larger amounts the proportion of Heterophyllus decreases with age of seed, and Pseudowhiptail and Poorkin increase. Balloon Rosette and Flat Rosette remain about the same or decline slightly to extensively in subsequent plantings. While selection does not always have the expected effect, the largest proportions of any type usually result from selection.

YASUZUMI, GONPACHIRO. A Study on Spermatogenesis in Animals with the Electron Microscope. *Laboratory for Electron Microscope Research, Department of Anatomy, Nara Medical College, Kashihara, Japan.*

The submicroscopic structure of such maturing spermatids as *Gelastorrhinus bicolor* de Haan, *Cipangopaludina malleata,* and *Eriocheir japonicus* de Haan has been studied in thin tissue sections by electron microscopy. As development proceeds, the nuclei of the spermatids undergo greater changes which affect both their forms and their internal fine structure. These investigations showed a regular arrangement of nuclear contents at an early stage of development and pronounced alteration of the arrangement during maturation. On the basis of evidence afforded by an electron microscopic examination as well as by cytological analysis, the atypical spermatozoa of *Cipangopaludina malleata* are to be interpreted as the olygopyrene or the apyrene.

The submicroscopic structure of mitochondria and the structural changes leading to the formation and evolution of the "nebenkern" of the spermatids have been observed in a grasshopper and a pond snail. The nebenkern derivatives apply themselves to the sheath of the middle-piece in a compact helicoidal fashion.

YERGANIAN, G., D. K. FORD, and T. H. YOSIDA. Survival of Deletions in Cultured (*in vivo* and *in vitro*) Normal and Malignant Cells of the Chinese Hamster, *Cricetulus griseus. Children's Cancer Research Foundation, Harvard Medical School, Boston, Mass., U.S.A.*, and *British Columbia Medical Research Institute, Vancouver, B.C., Canada.*

Spontaneous deletions, generally classed as lethal aberrations, survive and become incorporated in heteroploid *in vitro* and *in vivo* "normal" and malignant cells. Translocations, pericentric inversions and centromeric fractures forming telocentrics and "half-chromosomes" have arisen from chromosomes I, III, VIII, and XI.

The most aberrant element is the largest metacentric or chromosome I. To date, five new forms have been derived from this chromosome alone. Sites of preferential breakage along the short arm of colchicine-pretreated chromosome I are identical to those formed by chemical mutagens (Livingston and Yerganian, Genetics *41*:652).

The new chromosome type A, resulting from a deletion about one micron from the centromere, appeared in tumor CH-38MC and two strains of the C line of "normal" fibroblast after many cell generations. Type B, involving a deletion of the long arm about 1.7 microns from the centromere, proved to be deleterious to the sublime of CH-38MC in which it arose. Type F appeared in tumor 51sp, a spon-

taneous adenocarcinoma, and was characterized by an insertion of about 1.5 microns of non-homologous chromatin in the long arm, and a deletion along the short arm some 2 microns from the centromere. Types E and G were noted in tumor 53sp, a spontaneous fibrosarcoma, and involved breaks in the short arm 1.6 and 2.5 microns, respectively, from the centromere. The latter form appeared together with the type A chromosome in a 20-methylcholanthrene treated subline of "normal" fibroblast, during the course of advancing hyperdiploidy.

The persistence of certain deletions reflects alterations in the expected selection against deleterious mutations. Forces that prevail in the classic diploid state are gradually replaced by new factors resulting from continuous culture in defined media and/or genetically appropriate hosts.

YOSIDA, T. H. Chromosomal Alterations in Malignant Cells of the Rat. *National Institute of Genetics, Japan,* and *Children's Cancer Research Foundation, Boston, Mass., U.S.A.*

Normal somatic cells of the rat contain 42 chromosomes, including some rod-, J- and V-shaped elements. On the other hand, the tumor stem cells of the Yoshida sarcoma, MTK-sarcoma II and III and Hirosaki sarcoma contained 40 and 38 chromosomes including two and four large V-shaped elements.

From the karyological analysis of the tumor cells of seven original hepatomas which were induced by administration of azo dye, we found that some hepatomas did not contain a large V-shaped chromosome, while other tumors contained one large V-shaped. On the basis of the karyological analysis of these tumor cells, it is suggested that the large V-shaped chromosomes containing tumor cells originated from the translocation of some non-homologous chromosomes.

Recently we found new tumor cells of the Yoshida sarcoma which contained one large J- and two large V-shaped chromosomes. The large J-element is derived from the translocation of two J-shaped chromosomes. The new tumor cells gradually increased in number, as the transplant generation progressed. At the twentieth transplant generation a new sub-line of the Yoshida sarcoma was established. These studies suggest that tumor cells having new chromosome translocations show a more competitive ability in comparison to that of the original ones.

Present studies with the Chinese hamster may provide information concerning the mode of origin of such chromosomal anomalies.

YOSIDA, T. H. *See* YERGANIAN, G., *et al.*

YUASA, AKIRA, and KENJI FUJII. The Organization of the Nuclear Material in *Spirillum* sp. *Department of Biology, College of General Education, University of Tokyo, Tokyo, Japan.*

A *Spirillum* sp. was isolated from stagnant water and cultured purely in reproducible media.

There are great differences in the morphology of the spirilla in young cultures

according to the media used. Young spirilla show zig-zag or spiral structure within the cells when cultured in a peptone medium containing Ca-lactate, but not in a peptone medium without Ca-lactate. Aged cells showed the same morphological characters independent of the medium. This zig-zag or spiral structure shows positive nuclear reaction when stained with Giemsa stain or gentian violet solution. The spiral structure of the cytoplasm is related to the deposition of lipid granules. The nuclear material is present in granular state in young spirilla, having some relation to volutin or metachromatic granules. As the cultures develop, the nuclear material aggregates into longitudinal rods.

During cell division, the nuclear material which seems to exist with metachromatic granules is divided transversely, but the division of the nuclear material does not always synchronize with cell division which is also a transverse constriction and fission.

Cells of *Spirillum* sp. were cut into ultra-thin sections with glass knives following methacrylate-embedding at 50°C. The sections indicated the existence of a cell wall and cytoplasmic membrane. The nuclear material was distributed in the granular state at nuclear sites.

YURA, TAKASHI. Genetic Alteration of Pyrroline-5-carboxylate Reductase in *Neurospora*. *Department of Microbiology, Yale University, New Haven, Conn., U.S.A.*

The pyridine nucleotide dependent enzyme, pyrroline-5-carboxylate reductase, catalyzes the terminal step in the biosynthesis of proline in *Neurospora crassa*: the reduction of Δ^1-pyrroline-5-carboxylate to form proline. A proline-requiring mutant of *N. crassa*, strain 21863, is genetically blocked in this step of proline formation and shows a greatly diminished activity of the reductase. Therefore, a comparative study of the pyrroline-5-carboxylate reductase formed by the proline-requiring strain 21863 and of the reductase of several wild-type strains of *N. crassa* has been carried out. Using partially purified enzyme preparations, it was found that the reductase of the mutant strain has an activation energy three to four times greater than that of the wild-type strains. Furthermore, a significant difference was found between mutant and wild-type reductase preparations with respect to stability to heat (40°–60°C). The reductase of the mutant is more labile than that of the wild-type strains, the activation energy for heat inactivation being several times greater with wild-type strains. These differences which have been observed in activation energy and in heat stability appear to be associated with the characteristics of the proline locus (*prol-1*) and may well reflect differences between the formed enzymes since appropriate mixture experiments failed to show interaction between the enzyme preparations from the two sources. The reductase preparations from the mutant and wild-type strains are similar in other respects including stoichiometry, relative activity with the two pyridine nucleotides (TPNH and DPNH), substrate affinity, pH optimum, and in fractionation behavior. The implication of these findings to the problem of gene action will be discussed.

ZABAVI, A. *See* WAHRMAN, J.

ZALOKAR, MARKO. Primary Gene Product: Protein or RNA? *Department of Microbiology, Yale University, New Haven, Conn., U.S.A.*

Enzyme formation is under genetic control. DNA, an essential genetic component, is thought to carry information regarding enzyme synthesis. It is not known, however, whether genes can serve directly as sites of enzyme formation, or whether genes act in protein synthesis through an intermediary such as RNA, this latter substance being suggested by the fact that RNA and protein synthesis appear to be interrelated. Knowledge of the sites of formation of protein and RNA might thus shed a good deal of light on the problem of the primary action of genetic material. Evidence bearing on this problem has been obtained through the development of a technique which permits the centrifugation of living hyphae of *Neurospora crassa*. By this technique it is possible to stratify and separate microsomes, mitochondria and nuclei. Prior to centrifugation, mycelium was fed a radioactive amino acid (C^{14} proline or tritiated leucine) for short periods of time (1–16 minutes). The mycelium was then centrifuged, fixed, and the unincorporated precursors dissolved in cold trichloracetic acid. Autoradiographs were then made. The site of uptake of the amino acid was then determined by counting β-tracks emanating from the diverse particulate fractions. In a similar way the uptake of nucleotides (C^{14} and tritiated uridine) was studied. Results obtained to the present time tentatively suggest that in *Neurospora* proteins are formed mainly in microsomes, while ribonucleic acid appears first in the nuclei and then migrates into the microsomes. The implication of these results will be discussed in relation to the problem of gene action.

ZARUBAILO, T. Y. Transforming Spring Strains of Soft Wheat (*Triticum aestivum* L.) into Winter Strains by Means of Pre-Winter Sowing. *All-Union Institute of Animal Breeding, Moscow, U.S.S.R.*

From 1947 to 1956, experiments aimed at obtaining winter strains of wheat from spring varieties by means of pre-winter sowing were carried out in the town of Pushkin near Leningrad. In these experiments the progeny of individual plants belonging to 30 strains of soft spring wheat (*Triticum aestivum*) were used after checking for homogeneity (pure lines). Part of the seeds of each progeny (line) was annually sown from generation to generation in the pre-winter period (early October), while the remaining seeds were sown in spring for control. By the beginning of winter, the seeds sown in the pre-winter period had time to yield young sprouts.

Out of the 30 strains taken for the experiment, 8 proved to be unsuitable, while the remaining 22, sown in the pre-winter period, were checked to the seventh and ninth generations. All these varieties yielded winter strains of wheat, and in part of them changes of their morphological characteristics were observed. The control seeds of these varieties were sown in spring and steadily preserved their homogeneity both in morphological characteristics and in the length of the vegetation period.

In the course of the first three or four generations sown in the pre-winter period, no considerable increase in the frost resistance of the experimental plants was observed. It remained at an average level of about 10%. It is only in the fourth or fifth generation that the degree of hardiness considerably increased, reaching an

average of 40 to 50%. This shows that the increase in the hardiness of the experimental plants took place in the course of the experiment.

In the frost-resistant strains thus obtained, genuine winter wheat characteristics are weakly expressed. When sown in spring, these plants form ears without any vernalization at all, with a slight delay in comparison to the control plants ("double purpose type"), or do so after a comparatively short period of pre-sowing vernalization (which lasted about 25 days in our experiments). At the same time, they require a much longer day (prolongation of the light stage). Genuine winter wheat characteristics (with a long stage of vernalization) only develop in these strains when their seeds are sown at earlier dates, closer to the term when winter wheat varieties are generally sown in the given region. In our experiments this result was obtained when sowing was carried out in early September.

The winter strains of wheat obtained from spring varieties differ from the initial varieties in their increased capacity for accumulating soluble carbohydrates in their cell fluid during the autumn period. They accumulate these substances in the same quantities as those observed in winter wheat (14.18% as against 8.11% in spring varieties). This means that pre-winter sowing brings about certain changes in the biochemical processes which take place in the cells of the plant. Evidently the modifications in these processes form the basis of the hereditary changes which lead to the development of winter strains from spring varieties.

To convince ourselves that the development of winter strains from spring varieties was not merely the result of accidental spontaneous crosses between the controls and plants belonging to winter varieties, one of the experimental strains of spring wheat (Milturum 321) was placed in conditions of artificial isolation of the ears by enclosing them into cellophane containers. In the fourth generation the progeny of such autumn-sown plants which had been submitted to isolation in the course of three generations showed a high degree of frost resistance (70%). When sown in spring, these seeds yielded 22% of winter plants and 30% of plants of a semi-winter type.

ZELLE, MAX R. Studies of a Large Cell, Radiation Resistant, Possibly Polyploid Strain Derived from *Escherichia coli* B/r. *Cornell University, Ithaca, N.Y., U.S.A.*

Comparative studies of normal and polyploid bacterial strains would provide a more critical approach to elucidation of cytogenetic mechanisms in bacteria and of the role of nuclear damage in bacterial inactivation by radiations. A stable, large cell strain, P6, has been isolated following treatment of *Escherichia coli* 82/r (derived from *E. coli* B/r) with camphor vapors.

P6 has displayed a number of characteristics compatible with the hypothesis that it is a polyploid derivative of its parent 82/r. P6 has approximately three times the dry weight, DNA content and RNA content per cell. Counts of the number of discrete Feulgen-positive nuclear structures suggest that P6 may have 1.5 times as many nuclei per cell as 82/r. If DNA is considered to be exclusively nuclear, this would indicate that P6 is diploid. P6 is significantly more resistant to high energy radiations and whereas 82/r yields typical exponential survival curves, P6 displays sigmoidal inactivation curves. The inactivation kinetics are compatible with either an increased nuclear number or an increase in ploidy.

However, essentially similar induced mutation rates are observed in P6 and 82/r

for three different mutation systems, two of which presumably involve mutation from a dominant to recessive alleles. The large cell characteristic, most easily followed by the coarsely granular colony structure, segregates much like any unselected character in genetic crosses. The large cell segregants are radiation-resistant and have sigmoidal survival curves like P6. Critical genetic tests designed to detect heterozygous genotypes have not yet given definitive results but suggest that P6 is not polyploid. Rather, P6 may have undergone a mutation which increases the cell size, radiation resistance and, apparently, nuclear number. This tentative conclusion is supported by evidence that this large-cell mutation occurs spontaneously and at increasing rates after increasing exposures to ultraviolet.

ZELLER, J. H. *See* HETZER, H. O., *et al.*

ZHEBRAK, A. R. Polyploidy in Wheat. *Academy of Science of the Belorussian S.S.R., Moscow, U.S.S.R.*

In the genus *Triticum*, the species *T. monococcum* and *T. timopheevi* are isolated from the others. Hybrids were made between these two species and various other species, and then the chromosome number of the resulting hybrid was doubled by treating the dried seeds or the seedlings with colchicine. Hybrids of the types with 28 chromosomes, such as *T. durum* × *T. timopheevi*, and *T. polonicum* × *T. timopheevi* are sterile, but by doubling their chromosomes they become fertile. The hybrid *T. durum* × *T. polonicum*, also with 28 chromosomes, is normally fertile, and doubling the chromosome number in such a fertile hybrid, decreases its fertility 16 times.

The autopolyploids *T. durum* ($2n = 56$) and *T. timopheevi* ($2n = 56$) have low fertility and produce only 4–5 grains per ear. By crossing these autopolyploids the fertility of the hybrids is sharply increased, and as many as 30 grains per ear are produced. Amphidiploids formed from *T. timopheevi* crossed with all the 28 chromosome species do not segregate into the initial species, and have a low degree of variability, which variability increases when the hybrid is crossed with a third species, especially *T. turgidum* × *T. timopheevi* ($2n = 56$) × *T. vulgare* ($2n = 42$). From the offspring of these hybrids ($2n = 49$) we succeeded in isolating forms beyond the limits of the 3 parent species; primitive wheats and forms corresponding to *T. compactum*, as well as valuable types that according to their productivity and resistance to fungus diseases are higher than the standard kinds of wheat.

By crossing the amphidiploid, *T. vulgare* × *T. timopheevi* ($2n = 70$), with *T. vulgare*, sesquidiploids were formed which are strongly segregating and produce in their offspring new combinations of characters that can be regarded as new forms of species.

ZIELINSKI, QUENTIN. Spontaneous Tetraploidy in Pears, *Pyrus communis* Lim. *Oregon State College, Corvallis, Ore., U.S.A.*

Results are reported on twelve new spontaneous tetraploid pears, *Pyrus communis* Lim., discovered in Oregon. Data are presented on morphology of flower and fruits, chromosome determinations, fertility, breeding behavior and horticultural use.

APPENDIX*

DODSON, EDWARD O., and C. K. YU. Centrifugal Force as a Mutagenic Agent: Preliminary Report. *University of Ottawa, Ottawa, Canada.*

We have begun a study of the effects of centrifugal force upon the chromosomes of barley, alone and in combination with phenol. Preliminary observations upon root-tip mitosis have been made. Forces of less than 2,000 g do not produce detectable effects. When seed was centrifuged for 6 hrs. at 2,000 g, the chromatin was moderately clumped and some segmentation of prophase chromosomes occurred. Germination was 90%. Centrifugation at 2,000 g for 20 hrs. had similar effects upon mitosis, but germination was reduced to 75%. Centrifugation at 25,000 g for 6 hrs. resulted in more marked clumping, extensive segmentation of chromosomes, and some amitosis. Germination was reduced to 30%. The combined effect of phenol treatment and centrifugation is under investigation, but results are not yet available. Also, treated seed has been planted out, so that phenotypic effects can be investigated, but no results are available at this writing. (Supported by NRC of Canada grant A-735.)

GLOUSHTSHENKO, I. E. Some Regularities of Vegetative Hybridization of Plants. *Institute of Genetics, Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.*

Our experiments of many years as well as the experiments of other research workers of the U.S.S.R. have shown that by means of grafting one can obtain hybrid forms which are similar to those obtained by sexual hybridization.

During recent years analogical results have been obtained by many scientists in different countries (I. Sinoto in Japan, R. Glavinich in Yugoslavia, R. Frankel in the U.S.A., R. Georgieva in Bulgaria and others). P. Sopikoff and H. Koushner (U.S.S.R.), K. Brantanoff and N. Nesteroff (Bulgaria), M. Gashek (Czechoslovakia), J. Benoit, P. Leroy, K. and R. Vendrely (France) and other scientists have demonstrated that vegetative hybridization can also be observed in animal organisms.

On the other hand vegetative hybrids of plants and animals have some peculiarities which distinguish them from sexual hybrids. Some of the main distinctions are as follows. (1) Vegetative hybrids are characterized by heterosis which is transmitted to the subsequent generations. (2) The behavior of dominant and recessive characters of vegetative hybrids is not identical with their behavior in sexual hybridization; thus, for example, plants with recessive characters often produce offspring possessing dominant characters. (3) Mosaic forms which possess heterogeneous cells and tissues, heterogeneous fruits, etc., are encountered more often among vegetative hybrids than among sexual hybrids. Interspecific graftings significantly differ from intraspecific graftings. Interspecific graftings produce

*These abstracts were received too late for inclusion in the alphabetical series.

changed characters as a rule after the grafting is repeated in the following generations. Among the offspring there is a large amplitude of variation which exceeds the variations observed, the forms initially taken for grafting. The new forms obtained usually do not produce further variations in the subsequent generations.

As breeding practice shows vegetative hybridization is a means of creating new forms and of improving old varieties of agricultural plants. Vegetative hybridization experiments show that the characters of organisms can be inherited not only through the nuclei, but in many other ways as well.

LUBIMOVA, VERA FYODOROVNA. On the Origin and Inheritance of Certain New Characters in Wheat-Couchgrass Hybrids. *Main Botanical Garden of the Academy of Sciences of the USSR, Moscow, U.S.S.R.*

(1) In remote hybridizations, besides the hybrids in which the characters of the parental plants are combined, new forms appear with teratological changes and new formations which neither of the parents possessed.

(2) In working with wheat-couchgrass hybrids the following results were observed: (*a*) plants appeared in which the stamens were to some degree transformed into pistils (stamen-pistils); (*b*) multiple-pistil flowers developed possessing all three normally developed stamens but with 2–3 or more additional pistils; (*c*) after fertilization the additional pistils developed into grains. In some of the additional pistils there were two and sometimes three ovules instead of one and double embryo sacs from which twins developed.

(3) In crossing plants which possess multiple-pistil flowers with ordinary plants offspring often appear with stamen-pistils instead of stamens. These plants give rise to multiple-pistil flowers and also to forms that manifest a tendency towards polyembryony. (4) The property of forming multiple-pistil flowers and other new formations is transmitted as a recessive character. (5) The systematic selection of certain new formations makes it possible to fix and obtain constant forms bearing these characters.

(6) Abundance of nutritive substances, a short day, and moderate temperature during the initial development and differentiation of the ears lead to the fuller manifestation of these characters.

(7) A study of the origin and inheritance of the new formations gives a better understanding of the problems of phylogeny and speciation and also makes it possible to create new forms with useful properties.

OLENOV, J. M. On the Increase of Resistance to the Action of Insecticides. *Leningrad, U.S.S.R.*

The possibilities of increasing the resistance to the action of DDT have been studied on five populations of *Drosophila melanogaster*, two of them not having come into contact with DDT before. In the latter populations subthreshold doses during 30 generations had no effect, and selection by propagating the insects which did not come into contact with poison increased the resistance more than

a hundred times during 17 generations. These results show the inapplicability of the theory of direct adaptation as an explanation of the resistance of populations.

The average resistance of the populations which have undergone many years of action of DDT is hardly changed as a result of the continuous influx of immigrants, but by means of individual selection we managed to increase it sharply during 4–6 generations. Thus the activity of natural selection preceding the experiment is shown.

The resistance in all lines proved to be polygenically caused (and in one case the maternal effect was found). This is partly explained by the physiological complexity of the character in question. At the same time the effectiveness of the selection illustrates the great hereditary heterogeneity of natural populations.

F_1 crossings between the resistant and sensitive lines resemble resistant parents. Thus, this part of the mutant genes' action spectre does not undergo negative selection in nature.

The highest resistance exceeding 500–1,000 times the level of sensitive lines was obtained in hybrid at the interlinear crossing of stable individuals.

The long-lasting preservation of resistance in natural populations after the causative factor has been removed may be satisfactorily interpreted in the following way. Simultaneously with the increase of resistance in nature (but not with laboratory selection under inbreeding conditions) a selection tending to an increase in the general viability of resistant individuals takes place. The final result is the formation of highly viable populations preserving their characters after the removal of the causative agent. The possibility of getting such a result depends on the degree of isolation characteristic of the given species.

Polyakov, I. M. Experimental Study of Fertilization Selectivity in Plants and Some Problems of General Genetics. *Ukrainian Academy of Science, Ukrainian Institute of Plant Breeding, Selection and Genetics, Kiev, U.S.S.R.*

With the advent of new experimental methods proof has been obtained that the fertilization process is selective and we are now able to understand its physiological basis and to investigate certain established laws and regularities in the selectivity of fertilization. The experimental studies carried out by Polyakov and Mikhailova with various species of *Nicotiana* make it possible to differentiate the selectivity which is observed in the progamic phase of the fertilization process, and in the phase of genesis. A number of processes taking place in the progamic phase are also experimentally differentiated. They include: (*a*) the interaction of the pollen with the tissues of the pistil which take a different form in the various parts of the pistil; (*b*) the interaction between the components of the pollen mixture, taking place in the tissues of the pistil.

The phenomena of incompatibility observed in some intraspecific and interspecific crossings represent the same fertilization selectivity expressed in a more marked degree and yield important experimental material for the differential analysis of the phenomenon as a whole. The totality of experimental data indicates the impossibility of reducing the causes of selective fertilization to the unequal competitive capacity of the pollen tubes. The new physiological and biochemical data obtained on the fertilization process in flowering plants are helpful in finding the right approach to the understanding of the physiological basis of selectivity in fertilization.

In conformity with certain internal and external factors, certain laws governing the processes of selectivity in fertilization can be established: (*a*) dependence of the direction taken by selectivity in fertilization on the quantitative relations of the various components present in the mixture of pollen and their proportions; (*b*) dependence of the selectivity of fertilization on the age factor: (*c*) dependence of the selectivity in fertilization on the alimentation conditions of plants, etc. The knowledge of laws governing selectivity in fertilization opens up a possibility for using them in plant-breeding practice.

The general trend of selectivity processes in fertilization expresses the principle of historic affinity in sexual reproduction. Certain stages of intraspecific and interspecific differentiation are reflected to a greater or lesser degree in the selectivity of fertilization. In self- and cross-pollinated plants, fertilization selectivity has certain specific features. In the course of generations, such plants may undergo certain changes of attitude with regard to alien pollen and that of the plant itself (which is equivalent to a change in the direction of fertilization selectivity). Heterosis and fertilization selectivity are connected, and the teleological interpretation of this problem is inadmissible. In each specific crossing the combination of male and female gametes is not determined by chance, but by combined physiological, biochemical, anatomical and cytological factors. The general direction in which these factors exercise their influence is historically determined. It also displays dependences on internal and external factors, sometimes governed by definite laws.

RAJAN, S. S. The Evolution of Yellow Sarson, *Brassica campestris* var. *sarson*: A Self-compatible Race in a Self-incompatible Group. *Division of Botany, Indian Agricultural Research Institute, New Delhi, India.*

The mating system in all the ten chromosome species of the genus *Brassica* is controlled by self-incompatibility alleles. An exception to this is one of the cultivated races, yellow *sarson*, of the *campestris* group. This important Indian oilseed crop is not only self-compatible but also predominantly self-pollinated. Cytogenetic, linkage and self-incompatibility studies made from crosses between the self-compatible yellow *sarson* and self-incompatible *toria* and brown *sarson*, all of the *campestris* group, have rendered possible the formulation of a hypothesis regarding the evolution of the self-compatible race. Cytogenetic studies reveal that the differences between yellow *sarson* and *toria* are of the nature of chromosomal inversion. Genetic analysis of five characters closely associated with the mode of pollination indicates that four of these characters are controlled by genes showing two-factor interaction of the complementary type. Characters favouring self-pollination were recessive in three and dominant in two cases. Linkage studies showed all these five characters to be located on the same chromosome with recombination values ranging from 19.4 to 48.2%. Where the character favouring self-pollination was dominant, the phase of linkage was repulsion. Self-incompatibility studies showed that the self-compatible yellow *sarson* was cross-compatible with the self-incompatible *toria* or brown *sarson*, only as the mother parent. This unilateral crossability points the self-compatibility to be of the primary type rather than a secondary compatibility derived from a pre-existing self-incompatibility. But analysis of the filial generations from the crosses showed self-fertility to behave

as a simple Mendelian recessive and this leads to the conclusion that the self-compatibility of yellow *sarson* is in a locus independent of the self-incompatibility alleles.

From these observations the evolutionary history of yellow *sarson* could be visualised as follows. A distant dispersal of a few plants of the self-incompatible race from the northern climate (Himalayas) could have been the original source of material. A recessive mutation of a major gene, independent of the self-incompatibility allelic loci but inactivating them, conferred self-fertility on the genotype. Under natural selection genes favouring self-pollination accumulated and became closely linked with the help of a recombination suppressing mechanism like chromosomal inversions.

STANTON, WILFRED ROBERT. The Origin of the Races of Maize in West Africa. *Ministry of Agriculture, Northern Nigeria, Regional Research Station, Samari, Northern Nigeria.*

A number of hypotheses are prevalent concerning the origin and mode of spread of maize in the old world, but few are capable of support from experimental taxonomic study. In most areas waves of secondary introduction and subsequent hybridization have obscured the identity of the earliest type.

A survey of the maize types of West Africa supports the theory of a desert route (via Spain, Venice, Turkey, and Egypt) bringing in the "Early Caribbean" race and a sea route bringing in a second race direct from Brazil to the West African coast. Socio-ethnological factors have tended to preserve these races, particularly the original northern introduction, and it is only recently that extensive introgression has occurred. It is suggested that in areas north of the ninth parallel, maize types may be found which are more closely related to the early Columbian introduction than those of almost any other part of the Old World.

TSITSIN, N. V. The Creation of New Species and Forms of the Cereals by the Method of Distant Hybridization. *Main Botanical Garden of the USSR Academy of Sciences, Moscow, U.S.S.R.*

Distant hybridization is one of the most important factors in natural selection in the organic world. It is a factor in the creation of new varieties and species of plants and also important in the field of cattle breeding. The elaboration of this method gains great economic importance in the enrichment of the flora and fauna in our country and particularly in the development of new regions. One of the areas where this method has been applied has been the crossing of cultivated and native plants. The work on cereal crops has proved particularly fruitful.

Triticum × *Agropyron* using *A. glaucum, A. elongatum, A. junceum, A. trichophorum* and *A. repens* have yielded new species of forage grain and perennial wheat (*Triticum agropyrotriticum perenne*) as well as two varieties of winter wheat *T. vulgare* var. *alboramosum* and *T. vulgare* var. *rubroramosum.* Perennial and grain-forage wheats open up new horizons in the use of wheat not only for grain with a high content of albumin (more than 20%) but at the same time for

forage grass or hay with an albumin content equal to that of the grain of common soft wheat. In *Secale* × *Agropyron* a great deal of sterility is encountered in the F_1 generation. With the induction of polyploidy we are able to overcome this sterility and obtain fertile hybrids. Crosses involving *Triticum, Hordeum* and *Secale* with *Elymus arenarius* and *E. giganteus* are difficult to perform. Freeing the embryos from the seed coat and growing them on nutritive media allows for the recovery of F_1 plants. Hybrid plants have been recovered from the following crops: *Triticum* × *E. arenarius, Triticum* × *E. giganteus, Triticum* × *E. niollis, Hordeum* × *E. giganteus, Secale* × *E. arenarius, (Triticum* × *Agropyron)* × *E. arenarius* and finally *(Triticum* × *Agropyron)* × *E. giganteus.* Seeds (P_2) were recovered from F_1 plants *(Triticum* × *E. giganteus)* after their treatment with colchicine and with repeated pollinations.

TURBIN, N. V. New Data on the Biology of Fertilization of Seed Plants. *Academy of Science of Belorussian SSR. Minsk, Belorussia, U.S.S.R.*

In the experiments with various varieties of *Triticum vulgare* and *T. durum* it was found that under strict self-pollination (under isolation) a much worse result is obtained than under natural pollination, when the possibility of dusting the flower stigma by pollen of other plants is not excluded (cross-pollination). The percentage of fertilization is lower and the absolute weight of seeds falls in tests under isolation.

The analysis of control data has shown that the above-mentioned difference in fertilization is due to the existence in one of them and the absence in the other of the possibility of additional cross-pollination. Thorough observations on a large number of plants of many wheat varieties have shown that the structure and biology of flowering of wheat favours the effect of additional cross-pollination. The possible frequency of occurrence of cross-pollination may be judged on the basis of the established fact that the quantity of flowers of wheat that are fully open during the whole period of flowering is 60–90% of their total number. By an additional artificial pollination of wheat one observes a positive result, proving the existence of interaction between the pollen of the plant and foreign pollen. Similar data have been obtained in experiments with tomatoes and other self-pollinated plants.

As for rye and partly for other cross-pollinated plants it is ascertained that cross-pollination of emasculated flowers gives a worse result than pollination of non-emasculated plants, when interaction of self and foreign pollen is possible. The essential difference between wheat and rye as regards biology of pollination and fertilization consists in the sharp difference of these plants (wheat and rye) in the degree of their self-fertilization. The above mentioned results are especially striking in tests of inbreeding of plants and the application of additional pollination by heterogeneric pollen. For instance, in rye the setting of seeds increases in such tests by 10–12 times as compared with ordinary inbreeding.

It is established that the effect of heterogeneric co-pollination is observed only when using foreign pollen taken from species related to the mother plants. Positive results may be observed only when heterogeneric pollen germinates on the stigmas of experimental plants. The most probable mechanism of its action consists in the extraction of biologically active substances effecting the germination of pollen of mother species and in some cases strengthening the fertilization capacity of the latter.

WEINSTEIN, ALEXANDER. Did Nägeli Fail to Understand Mendel's Work? *Biological Laboratories, Harvard University, Cambridge, Massachusetts, U.S.A.*

Nägeli, it has been assumed, failed to understand Mendel's work, and finally forgot it when writing his book on heredity and evolution. But before Mendel wrote, Nägeli and other hybridists knew that the progeny of hybrids tend to split into three classes resembling respectively the hybrid parent and the two pure ancestors. Nägeli knew Naudin's work, and had himself discussed segregation, and had proposed a mathematical formula to test the rate of reversion to the pure form when each successive generation is crossed to the same pure ancestor. Nägeli also distinguished between internal qualities and external characters. (See Sitzungsberichte der königlichen bayerischen Akademie der Wissenschaften zu München, 1865, vol. 2, pp. 395–443; 1866, vol. 1, pp. 71–93, 93–127.) These facts may explain why Mendel wrote to Nägeli in the first place.

Nägeli's formula was rendered unnecessary by Mendel's evidence that reversion is complete in F_2. Nägeli objected that (1) Mendel's results might vary with environment, (2) Mendel's ratios were empirical, not rational, (3) heredity in other plants might differ from heredity in peas. Nägeli could have found support for these objections in Mendel's work. (1) Mendel's paper mentions variability in four of the seven segregating characters. (2) Ratios from individual plants, though averaging close to 3:1, varied between 32:1 and 24:13 (less than 2:1). (3) The subsequent failure to find segregation in *Hieracium* caused Mendel himself to admit that to "take rules deduced from observation of [pea] hybrids to be laws of hybridization" might be "erroneous" because "*Hieracium* hybrids show a behavior exactly opposite to those of *Pisum*." Nägeli's letters and notes show he understood Mendel's theory; for he proposed testing it by breeding extracted homozygotes, and for this purpose asked for the very plants a modern geneticist would have picked. That Nägeli forgot Mendel's pea experiments is unlikely because Nägeli had an unusually good memory; Nägeli's book, though not mentioning Mendel, shows some influence of Mendel's paper; and in a later work Nägeli cited Mendel's *Hieracium* experiments under the title of the paper on peas.

(Supported by a grant from the Commonwealth Fund.)

WELLS BINDERY INC.
WALTHAM, MASS.
JAN. 1966